EXHIBIT 3

(化妆品安全技术规范 - 2015 年版)

Safety and Technical Standards for Cosmetics (2015)

Translation of Chapter I overview and Chapter VI toxicological test methods

第一章 概述

1 范围

本规范规定了化妆品的安全技术要求，包括通用要求、禁限用组分要求、准用组分要求以及检验评价方法等。

本规范适用于中华人民共和国境内生产和经营的化妆品（仅供境外销售的产品除外）。

Chapter I overview

1 Range

This Standards specify the safety technical requirements of cosmetics, including general requirements, prohibited and restricted components requirements, approved components requirements, inspection and evaluation methods, etc.

This Standards are applicable to cosmetics produced and operated within the territory of the People's Republic of China (except products only for overseas sales).

2 术语和释义

下列术语和释义适用于本规范。

2.1 化妆品原料：化妆品配方中使用的成分。

2.2 化妆品新原料：在国内首次使用于化妆品生产的天然或人工原料。

2.3 禁用组分：不得作为化妆品原料使用的物质。

2.4 限用组分：在限定条件下可作为化妆品原料使用的物质。

2.5 防腐剂：以抑制微生物在化妆品中的生长为目的而在化妆品中加入的物质。

2.6 防晒剂：利用光的吸收、反射或散射作用，以保护皮肤免受特定紫外线所带来的伤害或保护产品本身而在化妆品中加入的物质。

2.7 着色剂：利用吸收或反射可见光的原理，为使化妆品或其施用部位呈现颜色而在化妆品中加入的物质，但不包括第三章表 7 中规定的染发剂。

2.8 染发剂：为改变头发颜色而在化妆品中加入的物质。

2 Terms and interpretation

The following terms and interpretation apply to this specification.

2.1 Cosmetic raw materials: ingredients used in cosmetic formula.

2.2 New cosmetics raw materials: natural or artificial raw materials first used in cosmetics production in China.

2.3 Prohibited components: substances that cannot be used as cosmetic raw materials.

2.4 Restricted components: substances that can be used as cosmetic raw materials under limited conditions.

2.5 Preservative: substance added to cosmetics for the purpose of inhibiting the growth of microorganisms in cosmetics.

2.6 Sunscreen: a substance added to a cosmetic product to protect the skin from specific ultraviolet rays or to protect the product itself by the absorption, reflection, or scattering of light.

2.7 Colorant: a substance added to a cosmetic to make the cosmetic or its application part appear color by using the principle of absorption or reflection of visible light, but excluding the hair dye specified in Table 7 of chapter III.

2.8 Hair dye: substance added to cosmetics to change the color of hair.

2.9 淋洗类化妆品：在人体表面（皮肤、毛发、甲、口唇等）使用后及时清洗的化妆品。

2.10 驻留类化妆品：除淋洗类产品外的化妆品。

2.11 眼部化妆品：宣称用于眼周皮肤、睫毛部位的化妆品。

2.12 口唇化妆品：宣称用于嘴唇部的化妆品。

2.13 体用化妆品：宣称用于身体皮肤（不含头面部皮肤）的化妆品。

2.14 肤用化妆品：宣称用于皮肤上的化妆品。

2.15 儿童化妆品：宣称适用于儿童使用的化妆品。

2.16 专业使用：在专门场所由经过专业培训的人员操作使用。

2.17 包装材料：直接接触化妆品原料或化妆品的包装容器材料。

2.18 安全性风险物质：由化妆品原料、包装材料、生产、运输和存储过程中产生或带入的，暴露于人体可能对人体健康造成潜在危害的物质。

2.9 Rinsing cosmetics: cosmetics that are ished in time after use on the surface of human body (skin, hair, nail, lips, etc.).

2.10 Resident cosmetics: cosmetics other than rinsing products.

2.11 Eye cosmetics: cosmetics claimed to be used for the skin around the eyes and eyelashes.

2.12 Lip cosmetics: cosmetics claimed to be used on the lips.

2.13 Body cosmetics: cosmetics claimed to be used for the skin of the body (excluding the skin of the head and face).

2.14 Skin cosmetics: cosmetics claimed to be used on the skin.

2.15 Children's cosmetics: cosmetics claimed to be suitable for children.

2.16 Professional use: it is operated and used by specially trained personnel in special places.

2.17 Packaging materials: packaging materials that directly contact cosmetics raw materials or cosmetics.

2.18 Safety risk substances: substances generated or brought in by cosmetics raw materials, packaging materials, production, transportation and storage, which may cause potential harm to human health when exposed to human body.

3 化妆品安全通用要求

3.1 一般要求

3.1.1 化妆品应经安全性风险评估，确保在正常、合理的及可预见的使用条件下，不得对人体健康产生危害。

3.1.2 化妆品生产应符合化妆品生产规范的要求。化妆品的生产过程应科学合理，保证产品安全。

3.1.3 化妆品上市前应进行必要的检验，检验方法包括相关理化检验方法、微生物检验方法、毒理学试验方法和人体安全试验方法等。

3.1.4 化妆品应符合产品质量安全有关要求，经检验合格后方可出厂。

3 General requirements for cosmetics safety

3.1 General requirements

3.1.1 Cosmetics shall be subject to safety risk assessment to ensure that under normal, reasonable and foreseeable conditions of use, they shall not cause harm to human health.

3.1.2 The production of cosmetics shall meet the requirements of the cosmetics production specifications. The production process of cosmetics should be scientific and reasonable to ensure product safety.

3.1.3 Necessary inspection shall be carried out before cosmetics are put on the market, including relevant physical and chemical inspection methods, microbial inspection methods, toxicology test methods and human safety test methods.

3.1.4 Cosmetics shall meet the relevant requirements of product quality and safety, and can be delivered only after passing the inspection.

3.2 配方要求

3.2.1 化妆品配方不得使用本规范第二章表 1 和表 2 所列的化妆品禁用组分。若技术上无法避免禁用物质作为杂质带入化妆品时，国家有限量规定的应符合其规定；未规定限量的，应进行安全性风险评估，确保在正常、合理及可预见的适用条件下不得对人体健康产生危害。

3.2.2 化妆品配方中的原料如属于本规范第二章表 3 化妆品限用组分中所列的物质，使用要求应符合表中规定。

3.2.3 化妆品配方中所用防腐剂、防晒剂、着色剂、染发剂，必须是对应的本规范第三章表 4 至表 7 中所列的物质，使用要求应符合表中规定。

3.3 微生物学指标要求

化妆品中微生物指标应符合表 1 中规定的限值。

3.2 Formula requirements

3.2.1 The prohibited components of cosmetics listed in Table 1 and table 2 of the second chapter of this specification shall not be used in cosmetics formula.

If it is technically impossible to avoid prohibited substances being brought into cosmetics as impurities, the national limited provisions shall be complied with; If no limit is specified, safety risk assessment shall be carried out to ensure no harm to human health under normal, reasonable and foreseeable applicable conditions.

3.2.2 If the raw materials in the cosmetic formula belong to the substances listed in Table 3 of Chapter II of this specification, the use requirements shall meet the provisions in the table.

3.2.3 Preservatives, sunscreens, colorants and hair dyes used in the cosmetics formula must be the substances listed in tables 4 to 7 in Chapter III of this specification, and the use requirements shall meet the regulations in the table.

3.3 Microbiological index requirements

Microbiological indicators in cosmetics shall meet the limits specified in Table 1.

表 1 化妆品中微生物指标限值

微生物指标	限值	备注
菌落总数（CFU/g 或 CFU/ml）	≤500	眼部化妆品、口唇化妆品和儿童化妆品
	≤1000	其他化妆品
霉菌和酵母菌总数（CFU/g 或 CFU/ml）	≤100	
耐热大肠菌群/g（或 ml）	不得检出	
金黄色葡萄球菌/g（或 ml）	不得检出	
铜绿假单胞菌/g（或 ml）	不得检出	

Table 1 Limit values of microbiological indicators in cosmetics

Microbial index	Limit value	Remarks
Total number of colonies (CFU / g or CFU / ml)	≤ 500	Eye cosmetics, lip cosmetics
	≤ 1000	children's cosmetics
Total number of mold and yeast (CFU / g or CFU / ml)	≤ 100	other cosmetics
Thermotolerant coliforms / g (or ml)	shall not be detected	
Staphylococcus aureus / g (or ml)	shall not be detected	
Pseudomonas aeruginosa / g (or ml)	shall not be detected	

3.4 有害物质限值要求

化妆品中有害物质不得超过表 2 中规定的限值。

表 2 化妆品中有害物质限值

有害物质	限值（mg/kg）	备注
汞	1	含有机汞防腐剂的眼部化妆品除外
铅	10	
砷	2	
镉	5	
甲醇	2000	
二噁烷	30	
石棉	不得检出*	

3.4 Limit requirements for hazardous substances

Hazardous substances in cosmetics shall not exceed the limits specified in Table 2.

Table 2 limits of harmful substances in cosmetics

Hazardous substances	Limit (mg / kg)	Remarks
mercury	1	Except for eye cosmetics containing organomercury preservatives
lead	10	
arsenic	2	
cadmium	5	
methanol	2000	
Dioxane	30	
asbestos	No detection*	

3.5 包装材料要求

直接接触化妆品的包装材料应当安全，不得与化妆品发生化学反应，不得迁移或释放对人体产生危害的有毒有害物质。

3.6 标签要求

3.6.1 凡化妆品中所用原料按照本技术规范需在标签上标印使用条件和注意事项的，应按相应要求标注。

3.6.2 其他要求应符合国家有关法律法规和规章标准要求。

3.7 儿童用化妆品要求

3.7.1 儿童用化妆品在原料、配方、生产过程、标签、使用方式和质量安全控制等方面除满足正常的化妆品安全性要求外，还应满足相关特定的要求，以保证产品的安全性。

3.7.2 儿童用化妆品应在标签中明确适用对象。

3.5 Packaging material requirements

Packaging materials in direct contact with cosmetics shall be safe, shall not react with cosmetics, and shall not migrate or release toxic and harmful substances harmful to human body.

3.6 Label requirements

3.6.1 If the raw materials used in cosmetics need to be marked with the use conditions and precautions according to this technical specification, they shall be marked according to the corresponding requirements.

3.6.2 Other requirements shall meet the requirements of relevant national laws, regulations and standards.

3.7 Cosmetic requirements for children

3.7.1 In addition to the normal safety requirements of cosmetics, children's cosmetics should also meet the relevant specific requirements in terms of raw materials, formula, production process, label, use mode and quality safety control, so as to ensure the safety of products.

3.7.2 Children's cosmetics should be clearly identified in the label.

3.8 原料要求

3.8.1 化妆品原料应经安全性风险评估，确保在正常、合理及可预见的使用条件下，不得对人体健康产生危害。

3.8.2 化妆品原料质量安全要求应符合国家相应规定，并与生产工艺和检测技术所达到的水平相适应。

3.8.3 原料技术要求内容包括化妆品原料名称、登记号（CAS 号和/或 EINECS 号、INCI 名称、拉丁学名等）、使用目的、适用范围、规格、检测方法、可能存在的安全性风险物质及其控制措施等内容。

3.8.4 化妆品原料的包装、储运、使用等过程，均不得对化妆品原料造成污染。

直接接触化妆品原料的包装材料应当安全，不得与原料发生化学反应，不得迁移或释放对人体产生危害的有毒有害物质。

对有温度、相对湿度或其他特殊要求的化妆品原料应按规定条件储存。

3.8.5 化妆品原料应能通过标签追溯到原料的基本信息（包括但不限于原料标准中文名称、INCI 名称、CAS 号和/或 EINECS 号）、生产商名称、纯度或含量、生产批号或生产日期、保质期等中文标识。

属于危险化学品的化妆品原料，其标识应符合国家有关部门的规定。

3.8.6 动植物来源的化妆品原料应明确其来源、使用部位等信息。

动物脏器组织及血液制品或提取物的化妆品原料，应明确其来源、质量规格，不得使用未在原产国获准使用的此类原料。

3.8.7 使用化妆品新原料应符合国家有关规定。

3.8 Raw material requirements

3.8.1 Cosmetic raw materials shall be subject to safety risk assessment to ensure that under normal, reasonable and foreseeable conditions of use, there is no harm to human health.

3.8.2 The quality and safety requirements of cosmetics raw materials shall comply with the relevant national regulations and be compatible with the level achieved by the production process and detection technology.

3.8.3 The technical requirements for raw materials include the name of cosmetic raw materials, registration number (CAS number and / or EINECS number, INCI name, Latin scientific name, etc.), purpose of use, scope of application, specifications, testing methods, potential safety risk substances and control measures.

3.8.4 The packaging, storage, transportation and use of cosmetics raw materials shall not cause pollution to cosmetics raw materials.

Packaging materials that directly contact cosmetic raw materials shall be safe, shall not have chemical reaction with raw materials, and shall not migrate or release toxic and harmful substances harmful to human body.

Cosmetics raw materials with temperature, relative humidity or other special requirements shall be stored according to the specified conditions.

3.8.5 Cosmetic raw materials shall be able to trace the basic information of raw materials (including but not limited to the Chinese name of raw material standard, inci name, CAS number and / or EINECS number), manufacturer name, purity or content, production batch number or production date, shelf life and other Chinese marks through labels.

The identification of cosmetics raw materials belonging to hazardous chemicals shall conform to the regulations of relevant national departments.

3.8.6 The raw materials of cosmetics from animals and plants should be clear about their sources, using parts and other information.

The source and quality specification of cosmetics raw materials for animal organ tissue and blood products or extracts shall be specified. Such raw materials not approved for use in the country of origin shall not be used.

3.8.7 The use of new cosmetic raw materials shall comply with the relevant regulations of the state.

第六章 毒理学试验方法

1 毒理学试验方法总则

General Principles

1 范围

本部分规定了化妆品原料及其产品安全性评价的毒理学检测要求。本部分适用于对化妆品原料及其产品的安全性评价。

Chapter VI Toxicological Test Methods

1 General principles of toxicological test methods

General Principles

1 Range

This part specifies the toxicological test requirements for the safety evaluation of cosmetics raw materials and products. This part is applicable to the safety evaluation of cosmetics raw materials and products.

2 化妆品原料的安全性评价的毒理学检测

2.1 评价原则

化妆品原料在正常以及合理的、可预见的使用条件下，不得对人体健康产生危害。

2.2 毒理学检测项目的选择原则

化妆品的新原料，一般需进行下列毒理学试验：

（1）急性经口和急性经皮毒性试验；

（2）皮肤和急性眼刺激性/腐蚀性试验；

（3）皮肤变态反应试验；

（4）皮肤光毒性和光敏感试验※（原料具有紫外线吸收特性需做该项试验）；

（5）致突变试验（至少应包括一项基因突变试验和一项染色体畸变试验）；

（6）亚慢性经口和经皮毒性试验；

（7）致畸试验；

（8）慢性毒性/致癌性结合试验；

（9）毒物代谢及动力学试验※；

（10）根据原料的特性和用途，还可考虑其他必要的试验。如果该新原料与已用于化妆品的原料化学结构及特性相似，则可考虑减少某些试验。

本规定毒理学试验为原则性要求，可以根据该原料理化特性、定量构效关系、毒理学资料、临床研究、人群流行病学调查以及类似化合物的毒性等资料情况，增加或减免试验项目。

*试验方法参照 GB7919-87 化妆品安全性评价程序和方法；
OECD 化学物质试验指南(OECD Guidelines for Testing of Chemicals)

2 Toxicological test for safety evaluation of cosmetic raw materials

2.1 Evaluation principle

Under normal, reasonable and foreseeable conditions of use, cosmetic raw materials shall not cause harm to human health.

2.2 Selection principle of toxicology test items

The following toxicological tests are generally required for the new raw materials of cosmetics:

(1) Acute oral and dermal toxicity test;

(2) Skin and acute eye irritation / corrosiveness test;

(3) Skin allergy test;

(4) Skin phototoxicity and light sensitivity test ※ (this test is required for raw materials with UV absorption characteristics);

(5) Mutagenicity test (including at least one gene mutation test and one chromosome aberration test);

(6) Subchronic oral and percutaneous toxicity tests;

(7) Teratogenesis test;

(8) Chronic toxicity / carcinogenicity combination test;

(9) Toxicant metabolism and kinetics test ※;

(10) Other necessary tests can be considered according to the characteristics and use of raw materials. If the chemical structure and characteristics of the new raw material are similar to those of the raw material used in cosmetics, some tests may be considered to be reduced.

The toxicology test in this regulation is a principle requirement, and the test items can be increased or reduced according to the physical and chemical characteristics of the raw material, quantitative structure-activity relationship, toxicology data, clinical research, population epidemiological investigation and toxicity of similar compounds.

*The test methods refer to GB7919-87 cosmetics safety evaluation procedures and methods; OECD guidelines for testing of chemicals

3 化妆品产品安全性评价的毒理学检测

3.1 评价原则

在一般情况下，新开发的化妆品产品在投放市场前，应根据产品的用途和类别进行相应的试验，以评价其安全性。

3.2 检测项目的选择原则

3.2.1 由于化妆品种类繁多，在选择试验项目时应根据实际情况确定。

3.2.2 每天使用的化妆品需进行多次皮肤刺激性试验，进行多次皮肤刺激性试验者不再进行急性皮肤刺激性试验，间隔 1 日或数日使用和用后冲洗的化妆品进行急性皮肤刺激性试验。

3.2.3 与眼接触可能性小的产品不需进行急性眼刺激性试验。

3 Toxicological test for safety evaluation of cosmetic products

3.1 Evaluation principle

In general, before the newly developed cosmetic products are put on the market, corresponding tests should be carried out according to the use and category of the products to evaluate their safety.

3.2 Selection principle of test items

3.2.1 Due to the wide variety of cosmetics, the test items should be selected according to the actual situation.

3.2.2 The cosmetics used every day need to undergo multiple skin irritation tests. Those who have conducted multiple skin irritation tests will no longer undergo the acute skin irritation test, and the cosmetics used and rinsed after use will undergo the acute skin irritation test at an interval of 1 day or several days.

3.2.3 It is not necessary to carry out acute eye irritation test for products with low possibility of eye contact.

2 急性经口毒性试验

Acute Oral Toxicity Test

1 范围

本规范规定了动物急性经口毒性试验的基本原则、要求和方法。本规范适用于化妆品原料安全性毒理学检测。

2 试验目的

急性经口毒性试验是评估化妆品原料毒性特性的第一步，通过短时间经口染毒可提供对健康危害的信息。试验结果可作为化妆品原料毒性分级和标签标识以及确定亚慢性毒性试验和其他毒理学试验剂量的依据。

2 Acute oral toxicity test

1 Range

This Standards specifies the basic principles, requirements and methods of animal acute oral toxicity test. This Standards is applicable to the safety toxicology test of cosmetic raw materials.

2 Test purpose

Acute oral toxicity test is the first step to evaluate the toxicity characteristics of cosmetic raw materials, which can provide information on health hazards through short-term oral exposure. The test results can be used as the basis for toxicity classification and labeling of cosmetic raw materials, as well as for determining the dose of subchronic toxicity test and other toxicological tests.

3 定义

3.1 急性经口毒性 acute oral toxicity

一次或在 24h 内多次经口给予实验动物受试物后，动物在短期内出现的健康损害效应。

3.2 经口 LD50 半数致死量 medium lethal dose

经口一次给予受试物后，引起实验动物总体中半数死亡的毒物的统计学剂量。以单位体重接受受试物的重量(mg/kg 或 g/kg)来表示。

4 试验的基本原则

以管饲法经口给予各试验组动物不同剂量的受试物，每组用一个剂量，染毒剂量的选择可通过预试验确定。染毒后观察动物的毒性反应和死亡情况。试验期间死亡的动物要进行尸检，试验结束时仍存活的动物要处死并进行尸检。本方法主要适用于啮齿类动物的研究，但也可用于非啮齿类动物的研究。

3 Definition

3.1 Acute oral toxicity

The effect of health damage in a short period of time after one or more times of oral administration of test substance in 24 hours.

3.2 Medium lethal dose

The statistical dose of the toxicant that causes half of the total deaths of the experimental animals after one oral administration of the test substance. The weight of the test substance received per unit weight (mg / kg or g / kg).

4 Basic principles of test

Different doses of test substance are given orally by tube feeding method to each experimental group. Each group is given one dose. The selection of dose could be determined by pre-test. The toxicity and death of the animals are observed. The animals that died during the experiment should be autopsied, and the animals that survived at the end of the experiment should be executed and autopsied. This method is mainly suitable for the study of rodents, but it can also be used for the study of non-rodents.

5 试验方法

5.1 受试物

受试物应溶解或悬浮于适宜的介质中，建议首选水，其次是植物油(如玉米油)，或考虑使用其他介质（如羧甲基纤维素、明胶、淀粉等）。对非水溶性介质，应了解其毒理特性，否则应在试验前先确定其毒性。每次经口染毒液体的最大容量取决于实验动物的

大小，对啮齿类动物所给液体容量一般为 1mL/100g， 水溶液可至 2mL/100g。通过调整受试物溶液浓度使各剂量组经口染毒的容量一致。

5.2 实验动物和饲养环境

首选健康成年大鼠和小鼠，也可选用其他敏感动物。使用雌性动物应是未孕和未曾产仔的。实验动物体重之间相差不得超过平均体重的 20%。试验前动物要在实验动物房环境中至少适应 3—5d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

The test substance should be dissolved or suspended in a suitable medium. Water is the prior choice, followed by vegetable oil (such as corn oil), or other medium (such as carboxymethylcellulose, gelatin, starch, etc.). For non-water soluble media, the toxicological characteristics should be understood, otherwise the toxicity should be determined before the test. The maximum volume of liquid for each oral exposure depends on the size of experimental animals. The volume of liquid for rodents is generally 1ml / 100g, and the volume of water solution can reach 2ml / 100g. By adjusting the concentration of the test solution, the volume of each dose group is the same.

5.2 Laboratory animals and feeding environment

Healthy adult rats and mice are prior choice, and other sensitive animals can also be selected. Female animals should not be pregnant and have never been given birth. The difference between the weight of experimental animals shall not exceed 20% of the average weight. Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Standard compound feed is selected, and drinking water is not limited.

5.3 剂量水平

根据所选方法的要求，原则上应设 4—6 个剂量组，每组动物一般为 10 只，雌雄各半。

各剂量组间距大小以兼顾产生毒性大小和死亡为宜，通常以较大组距和较少量动物进行预试。如果受试物毒性很低，也可采用一次限量法，即用 10 只动物（雌雄各半）口服 5000mg/kg 体重剂量，当未引起动物死亡，可考虑不再进行多个剂量的急性经口毒性试验。

5.3 Dose level

According to the requirements of the selected method, 4-6 dose groups should be set up in principle, with 10 animals in each group, half male and half female.

The distance of each dose group is suitable for both toxicity and death, and the larger group distance and the smaller number of animals are usually used for pre test. If the toxicity of the test substance is very low, the one-time limit method can also be used, that is to say, 10 animals (half male and half female) can be used to orally take 5000mg / kg body weight dose. When the animal does not die, it can be considered not to carry out multiple doses of acute oral toxicity test.

5.4 试验步骤

5.4.1 试验前，实验动物禁食过夜，不限制饮水。若采用代谢率高的其他动物，禁食时间可以适当缩短。

5.4.2 正式试验时，称量动物体重，随机分组，然后对各组动物用管饲法一次进行染毒，若估计受试物毒性很低，一次给予容量太大，也可在 24h 内分 2—3 次染毒，但合并作为一次剂量计算。染毒后继续禁食 3 h—4 h。若采用分批多次染毒，根据染毒间隔长短，必要时可给动物一定量的食物和水。

5.4.3 染毒后，对每只动物都应有单独全面的记录，染毒第 1 d 要定时观察实验动物的中毒表现和死亡情况，其后至少每天进行一次仔细的检查。详细记录被毛和皮肤、眼睛和粘膜，呼吸、循环、自主神经和中枢神经系统、肢体活动和行为等改变。特别注意是否出现震颤、抽搐、流涎、腹泻、嗜睡和昏迷等症状。应记录毒作用体征出现和消失的时间和死亡时间。

5.4 Test procedure

5.4.1 Before the experiment, the experimental animals are fasted overnight, but drinking water is not restricted. If other animals with high metabolic rate are used, the fasting time can be shortened appropriately.

5.4.2 In the formal test, the animals are weighed and randomly divided into groups. Then the animals in each group are exposed to the drug once by tube feeding method. If the toxicity of the test substance is estimated to be very low and the capacity of one-time administration is too large, it can also be exposed to the drug 2-3 times in 24 hours, but it is combined as one-time dose calculation. Fasting continues for 3-4 hours. If it is used for multiple times in batches, a certain amount of food and water can be given to the animals if necessary according to the length of poisoning interval.

5.4.3 After poisoning, each animal shall be recorded separately and comprehensively. On the first day of poisoning, the poisoning performance and death of experimental animals shall be regularly observed, and then at least one careful inspection shall be conducted every day. The changes of hair and skin, eyes and mucous membrane, respiration, circulation, autonomic and central nervous system, limb activity and behavior are recorded in detail. Pay special attention to the symptoms of tremor, convulsion, salivation, diarrhea, drowsiness and coma. The time of appearance and disappearance of signs of toxic action and the time of death should be recorded.

5.4.4 观察期限一般不超过 14d，但观察时间并非一成不变，要视动物中毒反应的严重程度、症状出现快慢和恢复期长短而定。若有死亡延迟迹象，可延长观察时间。

观察期内存活动物每周称重，观察期结束存活动物应称重，处死后进行尸检。

5.4.5 对实验动物进行大体解剖学检查，并记录全部大体病理改变。对死亡和存活 24h 和 24h 以上动物并存在大体病理改变的器官应进行病理组织学检查。

5.4.6 可采用多种方法测定 LD50，建议采用一次最大限度试验、霍恩氏法、上-下法、概率单位-对数图解法和寇氏法等。

5.5 试验结果评价

评价试验结果时，应将 LD50 与观察到的毒性效应和尸检所见相结合考虑，LD50 值是受试物毒性分级和标签标识以及判定受试物经消化道摄入后引起动物死亡可能性大小的依据。引用 LD50 值时一定要注明所用实验动物的种属、性别、染毒途径、观察期限

等。评价应包括动物接触受试物与动物异常表现（包括行为和临床改变、大体损伤、体重变化、致死效应及其他毒性作用)的发生率和严重程度之间的关系。

毒性分级见表 1。

5.4.4 Generally, the observation period is not more than 14 days, but the observation time is not invariable. It depends on the severity of animal poisoning reaction, the speed of symptoms and the length of recovery period. If there are signs of death delay, the observation time can be prolonged.

During the observation period, the active objects are weighed every week, and the living animals should be weighed at the end of the observation period, and then the autopsy is performed.

5.4.5 The gross anatomy of experimental animals was examined and all gross pathological changes were recorded. Pathologic examination should be carried out on organs that have died and survived for 24 hours or more and have gross pathological changes.

5.4.6 LD50 can be determined by many methods. It is suggested to use one maximum test, horn's method, up-down method, probability unit logarithm diagram method and Coriolis method.

5.5 Evaluation of test results

When evaluating the test results, LD50 should be considered in combination with the observed toxic effects and autopsy findings. LD50 value is the basis of toxicity classification and label identification of the test substance, as well as the determination of the possibility of animal death caused by ingestion of the test substance through the digestive tract.When quoting LD50 value, it is necessary to indicate the species, sex, route of exposure, observation period, etc. of the experimental animals used.The evaluation should include the relationship between the incidence and severity of the animal's exposure to the test substance and the animal's abnormal performance (including behavior and clinical changes, general injury, weight change, lethal effect and other toxic effects).

See Table 1 for toxicity classification.

表 1 经口毒性分级

LD_{50} (mg/kg)	毒性分级
≤50	高毒
51—500	中等毒
501—5000	低毒
>5000	实际无毒

6 试验结果的解释

通过急性经口毒性试验和 LD50 的测定可评价受试物的毒性。其结果外推到人类的有效性很有限。

Table 1 oral toxicity classification

LD50 (mg/kg)	Toxicity classification
Less than 50	Highly toxic
51—500	Moderate toxicity
501—5000	Low toxicity
>5000	Practically non-toxic

6 Interpretation of test results

The toxicity of the test substance can be evaluated by acute oral toxicity test and LD50 determination. The validity of extrapolation of the results to humans is very limited.

3 急性经皮毒性试验

Acute Dermal Toxicity Test

1 范围

本规范规定了动物急性皮肤毒性试验的基本原则、要求和方法。本规范适用于化妆品原料安全性毒理学检测。

2 试验目的

急性皮肤毒性试验可确定受试物能否经皮肤吸收和短期作用所产生的毒性反应，可为化妆品原料毒性分级和标签标识以及确定亚慢性毒性试验和其他毒理学试验剂量提供依据。

3 Acute percutaneous toxicity test

1 Range

This specification specifies the basic principles, requirements and methods of animal acute skin toxicity test. This specification is applicable to the safety toxicology test of cosmetic raw materials.

2 Test purpose

The acute skin toxicity test can determine whether the test substance can be absorbed by the skin and the toxic reaction produced by the short-term effect. It can provide the basis for the toxicity classification and labeling of cosmetics raw materials, as well as the determination of sub chronic toxicity test and other toxicological test doses.

3 定义

3.1 急性皮肤毒性 acute dermal toxicity

经皮一次涂敷受试物后，动物在短期内出现的健康损害效应。

3.2 经皮 LD50 半数致死量 mdium lethal dose

经皮一次涂敷受试物后，引起实验动物总体中半数死亡的毒物的统计学剂量。以单位体重涂敷受试物的重量(mg/kg 或 g/kg)来表示。

4 试验的基本原则

受试物以不同剂量经皮给予各组实验动物，每组用一个剂量。染毒后观察动物的毒性反应和死亡情况。试验期间死亡的动物要进行尸检，试验结束时仍存活的动物要处死并进行尸检。若已知受试物具有腐蚀性或强刺激性可不进行急性经皮毒性试验。

3 Definition

3.1 Acute dermal toxicity

The effect of health damage on animals in a short period of time after a single application of the test substance.

3.2 Medium lethal dose

The statistical dose of the toxicant that causes half of the total deaths in the experimental animals after a single skin application of the test substance. The weight of the coated test substance per unit weight (mg / kg or g / kg).

4 Basic principles of test

The test substance is given to each group of experimental animals in different doses through skin, with one dose for each group. The toxicity and death of the animals are observed. The animals that die during the experiment should be autopsied, and the animals that survived at the end of the experiment should be executed and autopsied. If the test substance is known to be corrosive or highly irritating, acute percutaneous toxicity test may not be carried out.

5 试验方法

5.1 受试物

液体受试物一般不需稀释。若受试物为固体，应研磨成细粉状，并用适量水或无毒、无刺激性、不影响受试物穿透皮肤、不与受试物反应的介质混匀，以保证受试物与皮肤有良好的接触。常用的介质有橄榄油、羊毛脂、凡士林等。

5.2 实验动物和饲养环境

可选用健康成年大鼠、家兔或豚鼠作为实验动物，也可使用其他种属动物进行试验。使用雌性动物应是未孕和未曾产仔的。建议实验动物体重范围为：大鼠 200g—300g；家兔 2kg—3kg；豚鼠 350g—450g。实验动物皮肤应健康无破损。试验前动物要在实验动物房环境中至少适应 3d—5d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

In general, the liquid test substance does not need to be diluted. If the test substance is solid, it shall be ground into fine powder, and mix with appropriate amount of water or non-toxic, non irritant medium, which does not affect the penetration of the test substance into the skin and does not react with the test substance, so as to ensure good contact between the test substance and the skin. Commonly used media are olive oil, lanolin, Vaseline, etc.

5.2 Laboratory animals and feeding environment

Healthy adult rats, rabbits or guinea pigs can be selected as experimental animals, or other species of animals can be used for the test. Female animals should not be pregnant and have not given birth. It is suggested that the weight range of experimental animals be: 200g-300g for rats, and 200g-300g for rabbits 2kg-3kg; 350g-450g for guinea pigs. The skin of experimental animals should be healthy without damage. Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Standard compound feed is selected, and drinking water is not limited.

5.3 剂量水平

根据所选用的方法要求，原则上应设 4—6 个剂量组，每组动物一般为 10 只，雌雄各半。各剂量组间距大小以兼顾产生毒性大小和死亡为宜，通常以较大组距和较少量动物进行预试。如果受试物毒性很低，可采用一次限量法，即用 10 只动物（雌雄各半）皮肤涂抹 2000mg/kg 体重剂量，当未引起动物死亡，可考虑不再进行多个剂量的急性经皮毒性试验。

5.3 Dose level

According to the requirements of the selected method, in principle, 4-6 dosage groups should be set up, each group of animals is generally 10 animals with half male and half female. The distance between different dosage groups is suitable for both toxicity and death. Usually, a larger group distance and a smaller number of animals are used for pre-test. If the toxicity of the tested substance is very low, a one-time limit method can be adopted, i.e. the skin of 10 animals (half male and half female) is smeared with a dose of 2000mg/kg body weight. When no animal death is caused, multiple doses of acute percutaneous toxicity tests can be considered no longer.

5.4 试验步骤

5.4.1 试验开始前 24h，剪去或剃除动物躯干背部拟染毒区域的被毛，去毛时应非常小心，不要损伤皮肤以免影响皮肤的通透性。涂皮面积约占动物体表面积的 10%，应根据动物体重确定涂皮面积。体重为 200g—300g 的大鼠约为 30cm2—40cm2，体重为 2kg—3kg 的家兔约为 160 cm2—210 cm2,体重为 350g—450g 的豚鼠约为 46 cm2—54 cm2。

5.4.2 将受试物均匀涂敷于动物背部皮肤染毒区，然后用一层薄胶片覆盖，无刺激胶布固定，防止动物舔食。若受试物毒性较高，可减少涂敷面积，但涂敷仍需尽可能薄而均匀。一般封闭接触 24h。

5.4.3 染毒结束后，应使用水或其他适宜的溶液清除残留受试物。

5.4.4 观察期限一般不超过 14d，但要视动物中毒反应的严重程度、症状出现快慢和恢复期长短而定。若有延迟死亡迹象，可考虑延长观察时间。

5.4 Test procedure

5.4.1 24 hours before the start of the test, cut or shave the coat of the area to be poisoned on the back of the animal trunk. When removing the coat, take great care not to damage the skin to avoid affecting the permeability of the skin. The coated area is about 10% of the surface area of the animal. The coated area should be determined according to the weight of the animal. The body weight of rats is about 30cm2-40cm2, that of rabbits is about 160cm2-210cm2, and that of guinea pigs is about 46cm2-54cm2.

5.4.2 The test substance shall be evenly applied to the toxic area of the skin on the back of the animal, and then covered with a thin film, fixed with non irritating adhesive tape, so as to prevent the animal from licking. If the toxicity of the test substance is high, the coating area can be reduced, but the coating should be as thin and uniform as possible. Generally, it is closed for 24 hours.

5.4.3 At the end of the exposure, water or other suitable solution shall be used to remove the residual test substance.

5.4.4 The duration of observation generally does not exceed 14 days, but depends on the severity of the animal poisoning reaction, the speed of symptoms and the length of recovery period. If there are signs of delayed death, extended observation time can be considered.

5.4.5 对每只动物都应有单独全面的记录，染毒第 1 d 要定时观察实验动物的中毒表现和死亡情况，其后至少每天进行一次仔细的检查。包括被毛和皮肤、眼睛和粘膜以及呼吸、循环、自主神经和中枢神经系统、肢体运动和行为活动等的改变。特别注意观察动物是否出现震颤、抽搐、流涎、腹泻、嗜睡、和昏迷等症状。死亡时间的记录应尽可能准确。

观察期内存活动物每周称重、观察期结束存活动物应称重，处死后进行尸检。

5.4.6 对实验动物进行大体解剖学检查，并记录全部大体病理改变。对死亡和存活 24h 和 24h 以上动物并存在大体病理改变的器官应进行病理组织学检查。

5.4.7 可采用多种方法测定 LD50，建议采用一次最大限度试验法、霍恩氏法、上-下法、概率单位-对数图解法和寇氏法等。

5.4.5 Each animal shall be recorded separately and comprehensively. The poisoning performance and death of the experimental animal shall be observed regularly on the first day after exposure, and then at least one careful inspection shall be carried out every day. It includes the changes of coat and skin, eyes and mucous membrane, respiration, circulation, autonomic and central nervous system, limb movement and behavior. Special attention shall be paid to observe whether the animals have tremor, convulsion, salivation, diarrhea, drowsiness, coma and other symptoms. The time of death should be recorded as accurately as possible.

During the observation period, the living animals should be weighed every week. After the end of the observation period, the autopsy should be carried out.

5.4.6 The gross anatomy of experimental animals was examined and all gross pathological changes were recorded. Pathologic examination should be carried out on organs that have died and survived for 24 hours or more and have gross pathological changes.

5.4.7 LD50 can be determined by many methods. It is suggested to use the method of one-time maximum test, horn's method, up-down method, probability unit logarithm diagram method and Coriolis method.

5.5 试验结果评价

评价试验结果时，应将经皮 LD50 与观察到的毒性效应和尸检所见相结合考虑，LD50 值是受试物毒性分级和标签标识以及判定受试物经皮肤吸收后引起动物死亡可能性大小的依据。引用 LD50 值时一定要注明所用实验动物的种属、性别、染毒途径、观察期限等。评价应包括动物接触受试物与动物异常表现（包括行为和临床改变、大体损伤、体重变化、致死效应及其他毒性作用）的发生率和严重程度之间的关系。

毒性分级见表 1。

5.5 Evaluation of test results

When evaluating the test results, we should consider the dermal LD50 combined with the observed toxic effects and autopsy findings. The LD50 value is the basis of the toxicity classification and label identification of the test substance and the determination of the possibility of animal death caused by the absorption of the test substance through the skin. When quoting LD50 value, it is necessary to indicate the species, sex, route of exposure, observation period, etc. of the experimental animals used. The evaluation should include the relationship between the incidence and severity of the animal's exposure to the test substance and the animal's abnormal performance (including behavior and clinical changes, general injury, weight change, lethal effect and other toxic effects).

See Table 1 for toxicity classification.

表1 皮肤毒性分级

LD_{50}（mg/kg）	毒性分级
＜5	剧毒
5—＜44	高毒
44—＜350	中等毒
350—＜2180	低毒
≥2180	微毒

6 试验结果的解释

急性经皮毒性试验研究和经皮 LD50 的确定提供了受试物经皮染毒的毒性。其结果外推到人类的有效性很有限。急性经皮毒性试验的结果应与经其他途径染毒的急性毒性试验结果相结合进行综合评价。

Table 1 skin toxicity classification

LD50（mg/kg）	Toxicity classification
＜5	Extremely toxic
5—＜44	Highly toxic
44—＜350	Moderate toxicity
350—＜2180	Low toxicity
≥2180	Micro toxicity

6 Interpretation of test results

The acute dermal toxicity test and the determination of dermal LD50 provided the toxicity of the test substance. The validity of extrapolation of the results to humans is very limited. The results of acute percutaneous toxicity test should be combined with the results of acute toxicity test of other routes for comprehensive evaluation.

4 皮肤刺激性/腐蚀性试验

Dermal Irritation/Corrosion Test

1 范围

本规范规定了动物皮肤刺激性或腐蚀性试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

确定和评价化妆品原料及其产品对哺乳动物皮肤局部是否有刺激作用或腐蚀作用及其程度。

4 Dermal Irritation/Corrosion Test

1 Range

This Test specifies the basic principles, requirements and methods of animal skin irritation or Corrosion test. This Test is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To determine and evaluate whether cosmetic raw materials and their products have irritating or corrosive effects on mammalian skin and their degree.

3 定义

3.1 皮肤刺激性 dermal irritation
皮肤涂敷受试物后局部产生的可逆性炎性变化。
3.2 皮肤腐蚀性 dermal corrosion
皮肤涂敷受试物后局部引起的不可逆性组织损伤。

4 试验的基本原则

将受试物一次(或多次)涂敷于受试动物的皮肤上，在规定的时间间隔内，观察动物皮肤局部刺激作用的程度并进行评分。采用自身对照，以评价受试物对皮肤的刺激作用。急性皮肤刺激性试验观察期限应足以评价该作用的可逆性或不可逆性。

动物如果在试验的任何阶段出现严重抑郁、痛苦的表现，则应当给予人道地处死。依据试验情况对受试物进行适当评价。

3 Definition

3.1 Dermal irritation
Reversible inflammatory changes in the local area of the skin after application of the test substance.
3.2 Dermal corrosion
Irreversible tissue damage caused by local application of the test substance on the skin.

4 Basic principles of test

The test substance is applied to the skin of the test animal once (or several times), and the degree of local skin irritation is observed and scored within the specified time interval. Self control is used to evaluate the skin irritation of the test substance. The

observation period of acute skin irritation test should be enough to evaluate the reversibility or irreversibility of the effect.

If animals show severe depression and pain at any stage of the trial, they should be executed humanely. The test object shall be evaluated properly according to the test situation.

5 试验方法

5.1 受试物

液体受试物一般不需稀释，可直接使用原液。若受试物为固体，应将其研磨成细粉状，并用水或其他无刺激性溶剂充分湿润，以保证受试物与皮肤有良好的接触。使用其他溶剂，应考虑到该溶剂对受试物皮肤刺激性的影响。需稀释后使用的产品，先进行产品原型的皮肤刺激性/腐蚀性试验，如果试验结果显示中度以上的刺激性，可按使用浓度为受试物再进行皮肤刺激性/腐蚀性试验。

受试物为强酸或强碱（pH 值 ≤2 或≥11.5），可以不再进行皮肤刺激试验。此外，若已知受试物有很强的经皮吸收毒性，经皮 LD50 小于 200mg/kg 体重或在急性经皮毒性试验中受试物剂量为 2000mg/kg 体重仍未出现皮肤刺激性作用，也无需进行急性皮肤刺激性试验。

5.2 实验动物和饲养环境

多种哺乳动物均可被选为实验动物，首选白色家兔。应使用成年、健康、皮肤无损伤的动物，雌性和雄性均可，但雌性动物应是未孕和未曾产仔的。实验动物至少要用 4 只，如要澄清某些可疑的反应则需增加实验动物数。实验动物应单笼饲养，试验前动物要在实验动物房环境中至少适应 3d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

Generally, the liquid test substance does not need to be diluted, and the original solution can be used directly. If the test substance is a solid, it shall be ground into a fine powder and moistened with water or other non irritant solvent to ensure good contact between the test substance and the skin. When using other solvents, the effect of the solvent on the skin irritation of the test object should be considered. For the products to be diluted and used, the skin irritation / corrosivity test of the product prototype shall be carried out first. If the test results show moderate or above irritation, the skin irritation / corrosivity test can be carried out according to the use concentration of the test substance.

If the test substance is strong acid or strong base (pH value ≤ 2 or ≥ 11.5), so skin irritation test can be stopped. In addition, if it is known that the test substance has a strong percutaneous absorption toxicity, the LD50 of the test substance is less than 200mg / kg body weight or the dose of the test substance is 2000mg / kg body weight in the acute percutaneous toxicity test, there is no need for the acute dermal irritation test.

5.2 Laboratory animals and feeding environment

A variety of mammals can be selected as experimental animals, white rabbits are the first choice. Adult, healthy and skin free animals should be used, both female and male, but female animals should not be pregnant and have not given birth. At least 4 experimental animals should be used. To clarify some suspicious reactions, the number of experimental animals should be increased. The experimental animals should be raised in single cage. Before the experiment, the animals should adapt to the environment of the laboratory for at least three days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, drinking water is not limited.

5.3 急性皮肤刺激性试验步骤

5.3.1 试验前约 24 h ，将实验动物背部脊柱两侧毛剪掉，不可损伤表皮，去毛范围左、右各约 3cm×3cm。

5.3.2 取受试物约 0. 5 mL（g）直接涂在皮肤上，然后用二层纱布（2.5cm×2.5cm）和一层玻璃纸或类似物覆盖，再用无刺激性胶布和绷带加以固定。另一侧皮肤作为对照。采用封闭试验，敷用时间为 4h。对化妆品产品而言，可根据人的实际使用和产品类型，延长或缩短敷用时间。对用后冲洗的化妆品产品，仅采用 2 h 敷用试验。试验结束后用温水或无刺激性溶剂清除残留受试物。

如怀疑受试物可能引起严重刺激或腐蚀作用，可采取分段试验，将三个涂布受试物的纱布块同时或先后敷贴在一只家兔背部脱毛区皮肤上，分别于涂敷后 3 min、60min 和 4 h 取下一块纱布，皮肤涂敷部位在任一时间点出现腐蚀作用，即可停止试验。

5.3.3 于清除受试物后的 1、24、48 和 72 h 观察涂抹部位皮肤反应，按表 1 进行皮肤反应评分，以受试动物积分的平均值进行综合评价，根据 24、48 和 72 h 各观察时点最高积分均值，按表 2 判定皮肤刺激强度。

5.3.4 观察时间的确定应足以观察到可逆或不可逆刺激作用的全过程，一般不超过 14d。

5.3 Acute skin irritation test procedure

5.3.1 About 24 hours before the experiment, the hair on both sides of the back spine of the experimental animal is cut off, and the epidermis could not be damaged. The hair removal range is about 3cm × 3cm on the left and 3cm on the right.

5.3.2 Take about 0.5ml (g) of the test substance and apply it directly to the skin, then cover it with two layers of gauze (2.5cm × 2.5cm) and a layer of cellophane or similar substance, then fix it with non irritating adhesive tape and bandage. The other side of the skin served as a control. The application time is 4 hours. For cosmetic products, the application time can be prolonged or shortened according to the actual use and product type of people. For the cosmetics washed after use, only 2 hours application test is used. At the end of the test, the residual test substance is removed with warm water or non irritant solvent.

If it is suspected that the test substance may cause serious irritation or corrosion, a sectional test can be carried out. Three gauze blocks coated with the test substance can be applied to the depilated area skin of a rabbit's back at the same time or successively. One gauze can be removed at 3 min, 60 min and 4 h after the application respectively. The test can be stopped if there is corrosion at any time point on the coated area of the skin.

5.3.3 Observe the skin reaction of the smear site at 1, 24, 48 and 72 hours after the test substance is removed, score the skin reaction according to table 1, comprehensively evaluate according to the average value of the integral of the test animals, and determine the skin stimulation intensity according to table 2 according to the highest integral mean value of each observation time point at 24, 48 and 72 hours.

5.3.4 The observation time should be enough to observe the whole process of reversible or irreversible stimulation, generally no more than 14 days.

5.4 多次皮肤刺激性试验步骤

5.4.1 试验前将实验动物背部脊柱两侧被毛剪掉，去毛范围各为 3cm×3cm，涂抹面积 2.5cm×2.5cm。

5.4.2 取受试物约 0.5mL(g)涂抹在一侧皮肤上，当受试物使用无刺激性溶剂配制时，另一侧涂溶剂作为对照，每天涂抹 1 次，连续涂抹 14d。从第二天开始，每次涂抹前应剪毛，用水或无刺激性溶剂清除残留受试物。一小时后观察结果，按表 1 评分，对照区和试验区同样处理。

5.4.3 结果评价：按下列公式计算每天每只动物平均积分，以表 2 判定皮肤刺激强度。

$$\text{每天每只动物平均积分} = \frac{\sum \text{红斑和水肿积分}}{\text{受试动物数}} \Big/ 14$$

5.4 Multiple skin irritation test procedures

5.4.1 Before the experiment, the two sides of the back spine of the experimental animal are cut off, the hair removal range is 3cm × 3cm, and the application area is 2.5cm×2.5cm。

5.4.2 Take about 0.5ml (g) of the test substance and apply it on one side of the skin. When the test substance is prepared with non irritant solvent, apply the solvent on the other side as a control, once a day for 14 days. From the next day, the hair shall be cut before each application, and the residual test substance shall be removed with water or non irritant solvent. One hour later, the observation results are scored according to table 1, and the control area and the test area are treated the same.

5.4.3 Results evaluation: calculate the average score of each animal every day according to the following formula, and determine the skin irritation intensity according to table 2.

Average score per animal per day = $\sum$ Erythema and edema integral/Number of animals tested /14

表 1 皮肤刺激反应评分

皮肤反应	积分
红斑和焦痂形成	
无红斑	0
轻微红斑（勉强可见）	1
明显红斑	2
中度—重度红斑	3
严重红斑（紫红色）至轻微焦痂形成	4
水肿形成	
无水肿	0
轻微水肿（勉强可见）	1
轻度水肿（皮肤隆起轮廓清楚）	2
中度水肿（皮肤隆起约 1mm）	3
重度水肿（皮肤隆起超过 1mm，范围扩大）	4
最高积分	8

表 2 皮肤刺激强度分级

积分均值	强度
0 — ＜ 0.5	无刺激性
0.5— ＜ 2.0	轻刺激性
2.0 — ＜ 6.0	中刺激性
6.0 — 8.0	强刺激性

6 试验结果的解释

急性皮肤刺激试验结果从动物外推到人的可靠性很有限。白色家兔在大多数情况下对有刺激性或腐蚀性的物质较人类敏感。若用其他品系动物进行试验时也得到类似结果，则会增加从动物外推到人的可靠性。试验中使用封闭式接触是一种超常的实验室条件下的试验，在人类实际使用化妆品过程中很少存在这种接触方式。

Table 1 skin irritation response score

Skin reaction	integral
Erythema and eschar formation	
No erythema	0
Slight erythema (barely visible)	1
Obvious erythema	2
Moderate to severe erythema	3

Severe erythema (purplish red) to slight eschar formation	4
Edema formation	
No edema	0
Slight edema (barely visible)	1
Mild edema (clear contour of skin bulge)	2
Moderate oedema (skin swelling about 1mm)	3
Severe edema (skin swelling more than 1mm, expanded range)	4
Highest integral	8

Table 2 classification of skin irritation intensity

Integral mean	Strength
0 — ＜ 0.5	Non irritant
0.5— ＜ 2.0	Light irritant
2.0 — ＜ 6.0	Moderate irritant
6.0 — 8.0	Strong irritant

6 Interpretation of test results

The reliability of extrapolation of acute skin irritation test results from animal to human is very limited. In most cases, white rabbits are more sensitive to irritant or corrosive substances than human beings. If similar results are obtained when testing with other strains of animals, it will increase the reliability of extrapolation from animals to humans. The use of closed contact in the experiment is an extraordinary laboratory test, which is rarely used in the actual use of cosmetics.

5 急性眼刺激性/腐蚀性试验

Acute Eye Irritation/Corrosion Test

1 范围

本规范规定了动物急性眼刺激性或腐蚀性试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

确定和评价化妆品原料及其产品对哺乳动物的眼睛是否有刺激作用或腐蚀作用及其程度。

3 定义

3.1 眼睛刺激性 eye irritation

眼球表面接触受试物后所产生的可逆性炎性变化。

3.2 眼睛腐蚀性 eye corrosion

眼球表面接触受试物后引起的不可逆性组织损伤。

5 Acute eye irritation / Corrosion test

1 Range

This Test specifies the basic principles, requirements and methods of animal acute eye irritation or corrosion test. This Test is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To determine and evaluate whether cosmetic raw materials and their products have irritating or corrosive effects on mammalian eyes and their degree.

3 Definition

3.1 Eye irritation

Reversible inflammatory changes on the surface of the eyeball after contact with the test substance.

3.2 Eye corrosion

Irreversible tissue damage caused by the contact of the eyeball surface with the test object.

4 试验的基本原则

受试物以一次剂量滴入每只实验动物的一侧眼睛结膜囊内，以未作处理的另一侧眼睛作为自身对照。在规定的时间间隔内，观察对动物眼睛的刺激和腐蚀作用程度并评分，以此评价受试物对眼睛的刺激作用。观察期限应能足以评价刺激效应的可逆性或不可逆性。

动物如果在试验的任何阶段出现严重抑郁、痛苦的表现，应当给予人道地处死，依据试验情况对受试物进行适当评价。动物出现角膜穿孔、角膜溃疡、角膜 4 分超过 48h、缺乏光反射超过 72h、结膜溃疡、坏疽、腐烂等情况，通常为不可逆损伤的症状，也应当给予人道地处死。

4 Basic principles of test

The test substance is dripped into conjunctival sac of one eye of each experimental animal in a single dose, and the untreated other eye is used as self-control. In the specified time interval, the degree of eye irritation and corrosion is observed and scored to evaluate the eye irritation of the test object. The duration of observation should be sufficient to evaluate the reversibility or irreversibility of the stimulus effect.

If the animal shows severe depression and pain at any stage of the test, it shall be executed humanely, and the test object shall be properly evaluated according to the test situation. Animals with corneal perforation, corneal ulcer, cornea 4 points over 48h, lack of light reflection over 72h, conjunctival ulcer, gangrene, rot and other conditions, usually irreversible injury symptoms, should also be given a humane execution.

5 试验方法

5.1 受试物

液体受试物一般不需稀释，可直接使用原液，染毒量为 0.1mL。若受试物为固体或颗粒状，应将其研磨成细粉状，染毒量应为体积 0.1mL 或重量不大于 100mg（染毒量应进行记录）。

受试物为强酸或强碱（pH 值≤2 或≥11.5），或已证实对皮肤有腐蚀性或强刺激性时，可以不再进行眼刺激性试验。

气溶胶产品需喷至容器中，收集其液体再使用。

5.2 实验动物和饲养环境

首选健康成年白色家兔。至少使用 3 只家兔。试验前动物要在实验动物房环境中至少适应 3d 时间。在试验开始前的 24h 内要对试验动物的两只眼睛进行检查（包括使用荧光素钠检查）。有眼睛刺激症状、角膜缺陷和结膜损伤的动物不能用于试验。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

In general, the liquid test substance does not need to be diluted, and the original solution can be used directly, with a dose of 0.1ml. If the test substance is solid or granular, it shall be ground into fine powder, and the amount of poisoning shall be 0.1ml in volume or no more than 100mg in weight (the amount of poisoning shall be recorded).

When the test substance is strong acid or alkali (pH value ≤ 2 or ≥ 11.5), or it has been confirmed that it is corrosive or strong irritant to the skin, eye irritation test can be stopped.

Aerosol products need to be sprayed into containers to collect their liquid for reuse.

5.2 Laboratory animals and feeding environment

The first choice is healthy adult white rabbits. At least 3 rabbits are used. Before the experiment, the animals should adapt to the environment of the laboratory for at least three days. Two eyes of the test animal shall be examined within 24 hours before the start of the test (including the use of fluorescein sodium). Animals with eye irritation, corneal defects, and conjunctival damage should not be tested.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Standard compound feed is selected, and drinking water is not limited.

5.3 试验步骤

5.3.1 轻轻拉开家兔一侧眼睛的下眼睑，将受试物 0.1 mL（100mg）滴入(或涂入)结膜囊中，使上、下眼睑被动闭合 1s，以防止受试物丢失。另一侧眼睛不处理作自身对照。滴入受试物后 24h 内不冲洗眼睛。若认为必要，在 24h 时可进行冲洗。

5.3.2 若上述试验结果显示受试物有刺激性，需另选用 3 只家兔进行冲洗效果试验，即给家兔眼滴入受试物后 30s，用足量、流速较快但又不会引起动物眼损伤的水流冲洗至少 30s。

5.3.3 临床检查和评分：在滴入受试物后 1、24、48、72h 以及第 4d 和第 7d 对动物眼睛进行检查。如果 72 h 未出现刺激反应，即可终止试验。如果发现累及角膜或有其他眼刺激作用，7d 内不恢复者，为确定该损害的可逆性或不可逆性需延长观察时间，一般不超过 21d，并提供 7d、14d 和 21d 的观察报告。除了对角膜、虹膜、结膜进行观察外，其他损害效应均应当记录并报告。在每次检查中均应按表 1 眼损害的评分标准记录眼刺激反应的积分。

可使用放大镜、手持裂隙灯、生物显微镜或其他适用的仪器设备进行眼刺激反应检查。在 24h 观察和记录结束之后，对所有动物的眼睛应用荧光素钠作进一步检查。

5.3.4 对用后冲洗的产品（如洗面奶、发用品、育发冲洗类）只做 30s 冲洗试验，即滴入受试物后，眼闭合 1s，至第 30s 时用足量、流速较快但又不会引起动物眼损伤的水流冲洗 30s，然后按 5.3.3 进行检查和评分。

5.3.5 对染发剂类产品，只做 4s 冲洗试验，即滴入受试物后，眼闭合 1s，至第 4s 时用足量、流速较快但又不会引起动物眼损伤的水流冲洗 30s，然后按 5.3.3 进行检查和评分。

5.3 Test procedure

5.3.1 Gently open the lower eyelid of one eye of the rabbit, drop (or apply) 0.1ml (100mg) of the test substance into the conjunctival sac, make the upper and lower eyelids passively closed for 1s, so as to prevent the loss of the test substance. The other side of the eye is not treated as self-control. Do not wash eyes within 24 hours after dropping the test substance. If necessary, flush at 24 hours.

5.3.2 If the above test results show that the test substance is irritant, another 3 rabbits shall be selected for the flushing effect test, that is, 30 s after the test substance is dripped into the rabbit's eyes, and at least 30 s after the test substance is flushed with sufficient water with fast flow rate but without eye injury.

5.3.3 Clinical examination and score: the eyes of the animals are examined at 1, 24, 48, 72 hours, and at the 4th and 7th days after the infusion of the test substance. If there is no stimulation reaction in 72 hours, the test can be terminated. In case of corneal involvement or other eye irritation, the observation

time shall be extended to determine the reversibility or irreversibility of the damage, generally not more than 21 days, and the observation reports of 7 days, 14 days and 21 days shall be provided. In addition to the observation of cornea, iris and conjunctiva, other damage effects should be recorded and reported. In each examination, the score of eye irritation should be recorded according to the scoring standard of eye damage in Table 1.

Eye irritation can be examined with a magnifying glass, hand-held slit lamp, biomicroscope, or other suitable equipment. After 24 hours observation and recording, the eyes of all animals are further examined with fluorescein sodium.

5.3.4 For the products washed after use (such as facial cleanser, hair products and hair care washing), only 30 s washing test shall be conducted, i.e. after dropping into the test object, the eyes shall be closed for 1 s, and then washed for 30 s with sufficient water with fast flow rate but without eye injury of animals, and then the inspection and scoring shall be conducted according to 5.3.3.

5.3.5 For hair dye products, only 4-s washing test is conducted, i.e. after dropping into the test substance, the eyes are closed for 1 s, and then washed for 30 s with enough water with fast flow rate but without eye injury of animals, and then inspected and scored according to 5.3.3.

表 1 眼损害的评分标准

眼损害	积分
角膜：混浊（以最致密部位为准）无溃疡形成或混浊	0
散在或弥漫性混浊，虹膜清晰可见半透明区易分辨，虹膜模糊不清	1
出现灰白色半透明区，虹膜细节不清，瞳孔大小勉强可见	2
角膜混浊，虹膜无法辨认	3
	4
虹膜：正常	0
皱褶明显加深，充血、肿胀、角膜周围有中度充血，瞳孔对光仍有反应	1
出血、肉眼可见破坏，对光无反应（或出现其中之一反应）	2

结膜：充血（指睑结膜、球结膜部位）血管正常	0
血管充血呈鲜红色	1
血管充血呈深红色，血管不易分辨弥漫性充血呈紫红色	2 3
水肿	
无	0
轻微水肿（包括瞬膜）	1
明显水肿，伴有部分眼睑外翻	2

眼损害	积分
水肿至眼睑近半闭合	3
水肿至眼睑大半闭合	4

6 结果评价

化妆品原料—以给受试物后动物角膜、虹膜或结膜各自在 24、48 和 72h 观察时点的刺激反应积分的均值和恢复时间评价，按表 2 眼刺激反应分级判定受试物对眼的刺激强度。

Table 1 scoring standard of eye damage

Eye damage	integral
Cornea: turbid (subject to the densest part) without ulcer formation or turbidity	0
Scattered or diffuse turbidity, clear and translucent iris area, easy to distinguish, unclear iris	1 2
Gray white translucent area appears, iris details are unclear, pupil size is barely visible	3 4
The cornea is cloudy and the iris is illegible	

Iris: normal	0
The wrinkles are obviously deepened, hyperemia, swelling, moderate hyperemia around the cornea, and the pupil still responded to the light	1
Bleeding, visible damage, no response to light (or one of them)	2
Conjunctiva: normal blood vessels in hyperemia (palpebral conjunctiva and bulbar conjunctiva)	0
Blood vessel congestion is bright red	1
Blood vessel congestion is dark red, blood vessel is indistinguishable diffuse congestion is purplish red	2
edema	3
nothing	0
Mild edema (including blink film)	1
Obvious edema with partial ectropion	2

Eye damage	integral
Dropsy to near closure of eyelid	3
dropsy to greater closure of eyelid	4

6 Result evaluation

Cosmetic raw materials: the mean value and recovery time of the stimulus response scores of cornea, iris or conjunctiva at 24, 48 and 72 hours after the test substance is given to the animals, and the stimulus intensity of the test substance

to the eyes is determined according to the eye stimulus response classification in Table 2.

表 2 原料眼刺激性反应分级

可逆眼损伤	2A 级（轻刺激性） 2/3 动物的刺激反应积分均值：角膜浑浊≥1；虹膜≥1；结膜充血≥2；结膜水肿≥2 和上述刺激反应积分在≤7 天完全恢复 2B 级（刺激性） 2/3 动物的刺激反应积分均值：角膜浑浊≥1；虹膜≥1；结膜充血≥2；结膜水肿≥2 和上述刺激反应积分在＜21 天完全恢复
不可逆眼损伤	任 1 只动物的角膜、虹膜和/或结膜刺激反应积分在 21 天的观察期间没有完全恢复 2/3 动物的刺激反应积分均值：角膜浑浊≥3 和/或虹膜＞1.5

注：当角膜、虹膜、结膜积分为 0 时，可判为无刺激性，界于无刺激性和轻刺激性之间的为微刺激性。

化妆品产品—以给受试物后动物角膜、虹膜或结膜各自在 24、48 或 72h 观察时点的刺激反应的最高积分均值和恢复时间评价，按表 3 眼刺激反应分级判定受试物对眼的刺激强度。

表 3 产品眼刺激性反应分级

可逆损伤	微刺激性	动物的角膜、虹膜积分=0；结膜充血和/或结膜水肿积分≤2，且积分在＜7 天内降至 0
	轻刺激性	动物的角膜、虹膜、结膜积分在≤7 天降至 0
	刺激性	动物的角膜、虹膜、结膜积分在 8—21 天内降至 0
不可眼损伤	腐蚀性	①动物的角膜、虹膜和/或结膜积分在第 21 天时＞0 ,2/3 动物的眼刺激反应积分：角膜浑浊≥3 和/或虹膜=2

注：当角膜、虹膜、结膜积分为 0 时，可判为无刺激性。

7 试验结果的解释

急性眼刺激性试验结果从动物外推到人的可靠性很有限。白色家兔在大多数情况下对有刺激性或腐蚀性的物质较人类敏感。若用其他品系动物进行试验时也得到类似结果，则会增加从动物外推到人的可靠性。

Table 2 classification of eye irritation response of raw materials

Reversible eye injury	Class 2A (mild irritation) 2/3 The mean score of stimulus response in animals: corneal turbidity ≥ 1; iris ≥ 1; conjunctival hyperemia ≥ 2; conjunctival edema ≥ 2 and the above stimulus response score recovered completely in ≤ 7 days Grade 2B (irritant) 2/3 The mean score of stimulus response in animals: corneal turbidity ≥ 1; iris ≥ 1; conjunctival hyperemia ≥ 2; conjunctival edema ≥ 2 and the above stimulus response score recovered completely in < 21 days
Irreversible eye injury	1. The scores of corneal, iris and / or conjunctival irritation in any animal did not fully recover during the 21 day observation period 2. 2/3 The mean score of stimulus response in animals: corneal turbidity ≥ 3 and / or iris > 1.5

Note: When the score of cornea, iris and conjunctiva is 0, it can be judged as non irritant, and the one between non irritant and light irritant is micro irritant.

Cosmetic products - to evaluate the maximum integral mean value and recovery time of the stimulus response of the cornea, iris or conjunctiva of the animal at the observation time point of 24, 48 or 72 hours after the test object is given, and determine the stimulus intensity of the test object to the eye according to the eye stimulus response classification in Table 3.

Table 3 Classification of eye irritation response

Reversible eye injury	Microstimulation	The score of cornea and iris is 0; the score of conjunctival congestion and / or conjunctival edema is ≤ 2, and the score is < 0 in 7 days
	Light irritant	The scores of cornea, iris and conjunctiva of animals decreased to 0 at ≤ 7 days
	thrill	The scores of cornea, iris and conjunctiva of animals decreased to 0 in 8-21 days
Irreversible eye injury	corrosive	① The score of cornea, iris and / or conjunctiva in animals is > 0 on the 21st day ,2/3 The score of eye stimulation response in animals: corneal turbidity ≥ 3 and / or iris = 2

Note: when the score of cornea, iris and conjunctiva is 0, it can be judged as non irritant.

7 Interpretation of test results

The reliability of acute eye irritation test results extrapolated from animal to human is very limited. In most cases, white rabbits are more sensitive to irritant or corrosive substances than human beings. If similar results are obtained when testing with other strains of animals, it will increase the reliability of extrapolation from animals to humans.

6 皮肤变态反应试验

Skin Sensitisation Test

1 范围

本规范规定了动物皮肤变态反应试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

确定重复接触化妆品及其原料对哺乳动物是否可引起变态反应及其程度。

6 Skin Sensitisation test

1 Range

This specification specifies the basic principles, requirements and methods of animal skin allergy test. This specification is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To determine whether and to what extent repeated contact with cosmetics and their ingredients may cause allergic reactions to mammals.

3 定义

3.1 皮肤变态反应（过敏性接触性皮炎）skin sensitization，allergic contact dermatitis

是皮肤对一种物质产生的免疫源性皮肤反应。在人类这种反应可能以瘙痒、红斑、丘疹、水疱、融合水疱为特征。动物的反应不同，可能只见到皮肤红斑和水肿。

3.2 诱导接触 induction exposure

指机体通过接触受试物而诱导出过敏状态的试验性暴露。

3.3 诱导阶段 induction period

指机体通过接触受试物而诱导出过敏状态所需的时间，一般至少一周。

3.4 激发接触 challenge exposure

机体接受诱导暴露后，再次接触受试物的试验性暴露，以确定皮肤是否会出现过敏反应。

3 Definition

3.1 Skin sensitization (allergic contact dermatitis)

It is the skin's immune response to a substance. In humans, this response may be characterized by pruritus, erythema, papules, vesicles, and fusion vesicles. The reaction of animals is different, and only erythema and edema may be seen.

3.2 Induction exposure

It refers to the experimental exposure that the body induces the allergic state by contacting the test substance.

3.3 Induction period

It refers to the time required for the body to induce the allergic state by contacting the test substance, generally at least one week.

3.4 Challenge exposure

After the body receives the induced exposure, it is exposed to the test substance again to determine whether the skin will have allergic reaction.

4 试验的基本原则

实验动物通过多次皮肤涂抹（诱导接触）或皮内注射受试物 10d—14d（诱导阶段）后，给予激发剂量的受试物，观察实验动物并与对照动物比较对激发接触受试物的皮肤反应强度。

4.1 实验动物和饲养环境

一般选用健康、成年雄性或雌性豚鼠，雌性动物应选用未孕或未曾产仔的。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制，需注意补充适量 Vc。

4.2 动物试验前准备

试验前动物要在实验动物房环境中至少适应 3d—5d 时间。将动物随机分为受试物组和对照组，按所选用的试验方法，选择适当部位给动物去毛，避免损伤皮肤。试验开始和结束时应记录动物体重。

4.3 无论在诱导阶段或激发阶段均应对动物进行全面观察包括全身反应和局部反应，并作完整记录。

4.4 试验方法可靠性的检查

使用已知的能引起轻度/中度致敏的阳性物每隔半年检查一次。局部封闭涂皮法至少有

30%动物出现皮肤过敏反应；皮内注射法至少有 60%动物出现皮肤过敏反应。阳性物一般采用 2,4-二硝基氯代苯，肉桂醛，2-巯基苯并噻唑或对氨基苯酸乙酯。

4 Basic principles of test

After several times of skin application (induced contact) or intradermal injection of the test substance for 10-14 days (induction stage), the test substance is given with excitation dose. The skin reaction intensity of the test substance is observed and compared with that of the control animal.

4.1 Laboratory animals and feeding environment

Generally, healthy, adult male or female guinea pigs shall be selected, and female animals shall be those who are not pregnant or have not given birth.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, unlimited drinking water, and pay attention to supplement appropriate VC.

4.2 Preparation before animal test

Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days. The animals are randomly divided into test substance group and control group. According to the selected test method, the appropriate parts are selected to remove hair to avoid skin damage. Animal weight shall be recorded at the beginning and end of the test.

4.3 In both induction and stimulation stages, the animals should be observed comprehensively, including systemic and local reactions, and a complete record should be made.

4.4 Test method reliability check

Use known positive substances that can cause mild / moderate sensitization to check every six months. Local sealing coating method at least has

30% of the animals had skin anaphylaxis; at least 60% of the animals had skin anaphylaxis by intradermal injection.2,4-dinitrochlorobenzene, cinnamaldehyde, 2-Mercaptobenzothiazole or ethyl p-aminobenzoate are generally used as the positive substance.

5 试验方法

5.1 局部封闭涂皮试验（Buehler Test, BT）

5.1.1 动物数

试验组至少 20 只，对照组至少 10 只。

5.1.2 剂量水平

诱导接触受试物浓度为能引起皮肤轻度刺激反应的最高浓度，激发接触受试物浓度为不能引起皮肤刺激反应的最高浓度。试验浓度水平可以通过少量动物（2—3 只）的预试验获得。

水溶性受试物可用水或用无刺激性表面活性剂作为赋形剂，其他受试物可用 80%乙醇或丙酮等作赋形剂，并设溶剂对照。

5.1.3 试验步骤

5.1.3.1 试验前约 24 h ，将豚鼠背部左侧去毛，去毛范围为 4 cm2—6 cm2 。

5.1.3.2 诱导接触：将受试物约 0.2mL（g）涂在实验动物去毛区皮肤上，以二层纱布和一层玻璃纸覆盖，再以无刺激胶布封闭固定 6 h。第 7d 和第 14d 以同样方法重复一次。

5.1.3.3 激发接触：末次诱导后 14—28d，将约 0.2mL 的受试物涂于豚鼠背部右侧 2cm×2cm 去毛区（接触前 24h 脱毛），然后用二层纱布和一层玻璃纸覆盖，再以无刺激胶布固定 6h。

5.1.3.4 激发接触后 24h 和 48h 观察皮肤反应，按表 1 评分。

5.1.3.5 试验中需设阴性对照组，使用 5.1.3.2 和 5.1.3.3 的方法，在诱导接触时仅涂以溶剂作为对照，在激发接触时涂以受试物。对照组动物必须和受试物组动物为同一批。在实验室开展变态反应试验初期，或使用新的动物种属或品系时，需同时设阳性对照组。

5 test method

5.1 Buehler test (BT)

5.1.1 Number of animals

At least 20 in the experimental group and 10 in the control group.

5.1.2 Dose level

The concentration of induced contact test substance is the highest concentration that can cause mild skin irritation, and the concentration of induced contact test substance is the highest concentration that can not cause skin irritation.The test concentration level can be obtained by a small number of animals (2-3).

Water soluble test substance can be used as excipient with water or non irritant surfactant, other test substance can be used as excipient with 80% ethanol or acetone, etc., and solvent control is set.

5.1.3 Test procedure

5.1.3.1 About 24 hours before the experiment, the left side of the guinea pig's back is depilated in the range of 4-6 cm2.

5.1.3.2 Induced contact: Apply about 0.2ml (g) of the test substance on the skin of the depilated area of the experimental animal, cover it with two layers of gauze and one layer of cellophane, and then seal it with non irritating adhesive tape for 6 hours. The 7th and 14th day are repeated in the same way.

5.1.3.3 Excitation contact: at 14-28 days after the last induction, about 0.2ml of test substance is coated on the right side of guinea pig's back 2cm × 2cm Depilated area (depilated 24 hours before contact), then covered with two layers of gauze and one layer of cellophane, and then fixed with non irritating adhesive tape for 6 hours.

5.1.3.4 The skin reaction is observed 24 hours and 48 hours after stimulation, and the score is according to table 1.

5.1.3.5 The negative control group should be set up in the test. The methods of 5.1.3.2 and 5.1.3.3 should be used. Only the solvent should be used as the control when inducing contact and the test substance should be used when stimulating contact.The

control group animals must be the same batch as the test group animals.In the early stage of allergy test in the laboratory, or when using new animal species or strains, a positive control group should be set up at the same time.

表 1 变态反应试验皮肤反应评分

皮肤反应	积分
红斑和焦痂形成	
无红斑	0
轻微红斑（勉强可见）	1
明显红斑（散在或小块红斑）	2
中度—重度红斑	3
严重红斑（紫红色）至轻微焦痂形成	4
水肿形成	
无水肿	0
轻微水肿（勉强可见）	1
中度水肿（皮肤隆起轮廓清楚）	2
重度水肿（皮肤隆起约 1mm 或超过 1mm）	3
最高积分	7

Table 1 skin reaction score of allergy test

Skin reaction	integral
Erythema and eschar formation	
No erythema	0
Slight erythema (barely visible)	1
Marked erythema (scattered or small erythema)	2
Moderate to severe erythema	3
Severe erythema (purplish red) to slight eschar formation	4
Edema formation	
No edema	0
Slight edema (barely visible)	1
Moderate edema (clear contour of skin bulge)	2
Severe edema (skin swelling of about 1mm or more)	3

Highest integral	7

5.1.4 结果评价

5.1.4.1 当受试物组动物出现皮肤反应积分≥2 时，判为该动物出现皮肤变态反应阳性，按表 3 判定受试物的致敏强度。

5.1.4.2 如激发接触所得结果仍不能确定，应于第一次激发后一周，给予第二次激发，对照组作同步处理或按 5.2 的方法进行评价。

5.1.4 Result evaluation

5.1.4.1 When the skin reaction score of the animal in the subject group is ≥2, the animal is judged to be positive for skin allergy, and the sensitization intensity of the subject is judged according to Table 3.

5.1.4.2 If the result of excitation contact is still uncertain, the second excitation should be given one week after the first excitation, and the control group should be treated synchronously or evaluated according to the method of 5.2.

5.2 豚鼠最大值试验 (Guinea Pig Maximinatim Test , GPMT)

采用完全福氏佐剂 (Freund Complete Adjvant, FCA) 皮内注射方法检测致敏的可能性。

5.2.1 动物数

试验组至少用 10 只，对照组至少 5 只。如果试验结果难以确定受试物的致敏性，应增加动物数，试验组 20 只，对照组 10 只。

5.2.2 剂量水平

诱导接触受试物浓度为能引起皮肤轻度刺激反应的最高浓度，激发接触受试物浓度为不能引起皮肤刺激反应的最高浓度。试验浓度水平可以通过少量动物（2—3 只）的预试验获得。

5.2 Guinea Pig Maximinatim Test (GPMT)

The possibility of sensitization is detected by intradermal injection of Freund complete adjvant (FCA).

5.2.1 Number of animals

At least 10 in the experimental group and 5 in the control group. If the test results are difficult to determine the sensitization of the test substance, the number of animals should be increased, 20 in the test group and 10 in the control group.

5.2.2 Dose level

The concentration of induced contact test substance is the highest concentration that can cause mild skin irritation, and the concentration of induced contact test substance is the highest concentration that can not cause skin irritation. The test concentration level can be obtained by a small number of animals (2-3).

5.2.3 试验步骤

5.2.3.1 诱导接触（第 0d）

受试物组：将颈背部去毛区（2cm×4cm）中线两侧划定三个对称点，每点皮内注射 0.1mL 下述溶液。

第 1 点 1：1（V/V）FCA/水或生理盐水的
混合物第 2 点 耐受浓度的受试物
第 3 点 用 1：1（v/v）FCA/水或生理盐水配制的受试物，浓度与第 2
点相同对照组：注射部位同受试物组
第 1 点 1：1（V/V）FCA/水或生理盐水的
混合物第 2 点 未稀释的溶剂
第 3 点 用 1：1（v/v）FCA/水或生理盐水配制的浓度为 50%（w/v）的溶剂

5.2.3 Test procedure

5.2.3.1 Induced contact (0d)

Test substance group: draw three symmetrical points on both sides of the midline of the neck back hair removal area (2cm × 4cm), inject 0.1ml of the following solution into the skin at each point.

Point 1 1:1 (V / V) FCA / water or saline
mixture point 2 tolerance concentration of
test substance
Point 3: test substance prepared with 1:1 (V / V) FCA / water or
normal saline at the same concentration as point 2 control group:
injection site is the same as test substance group
Point 1 1:1 (V / V) FCA / water or saline
mixture point 2 undiluted solvent
Point 3 50% (w / V) solvent prepared with 1:1 (V / V) FCA / water or normal saline

5.2.3.2 诱导接触（第 7d）：

将涂有 0.5g(mL)受试物的 2cm×4cm 滤纸敷贴在上述再次去毛的注射部位，然后用两层纱布，一层玻璃纸覆盖，无刺激胶布封闭固定 48h。对无皮肤刺激作用的受试物，可加强致敏，于第二次诱导接触前 24h 在注射部位涂抹 10%十二烷基硫酸钠（SLS）0.5mL。对照组仅用溶剂作诱导处理。

5.2.3.3 激发接触（第 21d）

将豚鼠躯干部去毛，用涂有 0.5g(mL)受试物的 2cm×2cm 滤纸片敷贴在去毛区，然后再用两层纱布，一层玻璃纸覆盖，无刺激胶布封闭固定 24h。对照组动物作同样处理。如激发接触所得结果不能确定，可在第一次激发接触一周后进行第二次激发接触。对照组作同步处理。

5.2.3.2 Induced contact (day 7):

The 2cm × 4cm filter paper coated with 0.5g (ML) test substance is applied to the injection site where the hair is removed again, and then two layers of gauze and one layer of cellophane are used to cover the injection site, which is closed and fixed for 48h without stimulating adhesive tape. For the test substance without skin irritation, sensitization can be enhanced, and 0.5ml of 10% sodium dodecyl sulfate (SLS) can be applied to the injection site 24 hours before the second induced contact. The control group is treated with solvent only.

5.2.3.3 Excitation contact (21d)

Remove the hair from the trunk of guinea pig, apply the 2cm × 2cm filter paper coated with 0.5g (ML) test substance to the hair removal area, and then use two layers

of gauze, one layer of cellophane covered, sealed and fixed for 24h without stimulating adhesive tape. The control group is treated with the same method. If the result of excitation contact is uncertain, the second excitation contact can be carried out one week after the first excitation contact. The control group is treated synchronously.

5.2.4 观察及结果评价

激发接触结束，除去涂有受试物的滤纸后 24、48 和 72h，观察皮肤反应，（如需要清除受试残留物可用水或选用不改变皮肤已有反应和不损伤皮肤的溶剂）按表 2 评分。当受试物组动物皮肤反应积分≥1 时，应判为变态反应阳性，按表 3 对受试物进行致敏强度分级。

5.2.4 Observation and result evaluation

At the end of the stimulation contact, 24, 48 and 72 hours after removing the filter paper coated with the test substance, observe the skin reaction (if it is necessary to remove the test residue, use water or choose the solvent that does not change the existing skin reaction and does not damage the skin) according to table 2.When the skin reaction score of the animal in the test substance group is ≥ 1, it shall be judged as allergic reaction positive, and the test substance shall be graded according to the sensitization intensity in Table 3.

表 2 变态反应试验皮肤反应评分

评分	皮肤反应
0	未见皮肤反应
1	散在或小块红斑
2	中度红斑和融合红斑
3	重度红斑和水肿

Table 2 skin reaction score of allergy test

score	Skin reaction
0	No skin reaction
1	Scattered or small erythema
2	Moderate and confluent erythema
3	Severe erythema and edema

表 3 致敏强度

致敏率%	致敏强度
0—8	弱
9—28	轻
29— 64	中
65— 80	强
81—00	极强

注：当致敏率为 0 时，可判为未见皮肤变态反应。

Table 3 sensitization intensity

Sensitization rate%	Sensitization intensity
0 - 8	weak
9 - 28	light
29— 64	medium
65— 80	strong
81—00	Extremely strong

Note: When the sensitization rate is 0, it can be judged as no skin allergy.

6 试验结果的解释

试验结果应能得出受试物的致敏能力和强度。这些结果只能在很有限的范围内外推到人类。引起豚鼠强烈反应的物质在人群中也可能引起一定程度的变态反应，而引起豚鼠较弱反应的物质在人群中也许不能引起变态反应。

6 Interpretation of test results

The test results should be able to obtain the sensitization ability and strength of the test substance. These results can only be extrapolated to humans in a very limited range. The substances that cause the guinea pig's strong reaction may also cause allergic reaction to a certain extent in the crowd, while the substances that cause the guinea pig's weak reaction may not cause allergic reaction in the crowd.

7 皮肤光毒性试验

Skin Phototoxicity Test

1 范围

本规范规定了皮肤光毒性试验的基本原则，要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

评价化妆品原料及其产品引起皮肤光毒性的可能性。

7 Skin phototoxicity test

1 Range

This specification specifies the basic principles, requirements and methods of skin phototoxicity test. This specification is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To evaluate the possibility of skin phototoxicity caused by cosmetic materials and products.

3 定义

光毒性 phototoxicity

皮肤一次接触化学物质后，继而暴露于紫外线照射下所引发的一种皮肤毒性反应，或者全身应用化学物质后，暴露于紫外线照射下发生的类似反应。

4 试验的基本原则

将一定量受试物涂抹在动物背部去毛的皮肤上，经一定时间间隔后暴露于 UVA 光线下，观察受试动物皮肤反应并确定该受试物有否光毒性。

3 Definition

Phototoxicity

A skin toxic reaction caused by exposure of skin to chemicals at one time and then to ultraviolet radiation, or a similar reaction caused by exposure to ultraviolet radiation after application of chemicals in the whole body.

4 Basic principles of test

Apply a certain amount of test substance on the depilated skin on the back of the animal, after a certain time interval, expose to UVA light, observe the skin reaction of the test animal and determine whether the test substance has phototoxicity.

5 试验方法

5. 1 受试物

液体受试物一般不用稀释，可直接使用原液。若受试物为固体，应将其研磨成细粉状并用水或其他溶剂充分湿润，在使用溶剂时，应考虑到溶剂对受试动物皮肤刺激性的影响。对于化妆品产品而言，一般使用原霜或原液，所用受试物浓度不能引起皮肤刺激反应 (可通过预试验确定)。阳性对照物选用 8—甲氧基补骨脂 （8-methoxypsoralen，8—Mop）。

5. 2 实验动物和饲养条件

使用成年白色家兔或白化豚鼠，尽可能雌雄各半。选用 6 只动物进行正式试验。试验前动物要在实验动物房环境中至少适应 3d—5d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制，需注意补充适量 Vc。

5. 3 UV 光源

5.3.1 UV 光源：波长为 320nm—400nm 的 UVA，如含有 UVB，其剂量不得超过 0.1J/cm2。

5.3.2 强度的测定：用前需用辐射计量仪在实验动物背部照射区设 6 个点测定光强度

（mW/cm2），以平均值计。

5 test method

5. 1 Test substance

Generally, the liquid test substance does not need to be diluted, and the original solution can be used directly. If the test substance is a solid, it shall be ground into a fine powder and fully wetted with water or other solvents. When using solvents, the effects of solvents on the skin irritation of the test animals shall be taken into account. For cosmetic products, the original cream or solution is generally used, and the concentration of the test substance cannot cause skin irritation reaction (it can be determined by pre test).8-methoxypsoralen (8-MOP) is selected as the positive control.

5. 2 Laboratory animals and feeding conditions

Use adult white rabbits or albino guinea pigs, half male and half female as much as possible. Six animals are selected for formal test. Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, unlimited drinking water, and pay attention to supplement appropriate VC.

5.3 UV light source

5.3.1 UV light source: UVA with a wavelength of 320nm-400nm, if containing UVB, its dose shall not exceed 0.1j/cm2.

5.3.2 Determination of intensity: 6 points shall be set in the back irradiation area of experimental animals to measure the light intensity before use (MW / cm2), in average.

5.3.3 照射时间的计算：照射剂量为 10J/cm2，按下式计算照射时间。

$$照射时间(sec) = \frac{照射剂量(10000mJ/cm^2)}{光强度(mJ/cm^2/sec)}$$

注：$1\ mW/cm^2 = 1\ mJ/cm^2/sec$

5.3.3 Calculation of irradiation time: The irradiation dose is 10J/cm2, and the irradiation time is calculated according to the following formula.

Exposure time (sec) = irradiation dose (10000mJ/ cm2) / Light intensity (mJ/cm2/sec)

Note: 1 mW/cm2 = 1 mJ/cm2/sec

5.4 试验步骤

5.4.1 进行正式光毒试验前 18h—24h，将动物脊柱两侧皮肤去毛，试验部位皮肤需完好，无损伤及异常。备 4 块去毛区（见图 1），每块去毛面积约为 2cm×2cm。

5.4.2 将动物固定，按表 1 所示，在动物去毛区 1 和 2 涂敷 0.2mL(g)受试物，30min 后，左侧（去毛区 1 和 3）用铝箔复盖，胶带固定，右侧用 UVA 进行照射。

5.4.3 结束后分别于 1、24、48 和 72h 观察皮肤反应，根据表 2 判定每只动物皮肤反应评分。

5.4.4 为保证试验方法的可靠性，至少每半年用阳性对照物检查一次。即在去毛区 1 和 2 涂阳性对照物，方法同 5.4.2。

5.4 Test procedure

5.4.1 18-24 hours before the official phototoxicity test, the skin on both sides of the spine of the animal shall be depilated, and the skin at the test site shall be intact without damage or abnormality. Prepare 4 depilated areas (see Figure 1), each with an area of about 2cm × 2cm.

5.4.2 Fix the animal, as shown in Table 1, apply 0.2ml (g) of test substance to the depilated area 1 and 2 of the animal, 30 minutes later, cover the left side (depilated area 1 and 3) with aluminum foil, fix it with adhesive tape, and irradiate the right side with UVA.

5.4.3 The skin reaction is observed at 1, 24, 48 and 72 hours after the end of the treatment, and the skin reaction score of each animal is determined according to table 2.

5.4.4 In order to ensure the reliability of the test method, the positive control substance shall be checked at least once every six months. That is to say, positive control substances are applied to depilation areas 1 and 2 in the same way as in 5.4.2.

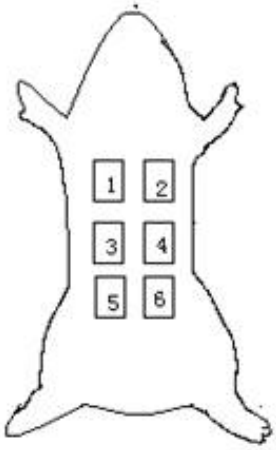


图 1 动物皮肤去毛区位置示意图

Fig. 1 location of hair removal area of animal skin

表 1 动物去毛区的试验安排

去毛区编号	试验处理
1	涂受试物，不照射
2	涂受试物，照射
3	不涂受试物，不照射
4	不涂受试物，照射

Table 1 experimental arrangement of animal hair removal area

Depilation Area No	Test treatment
1	Coated test substance, not irradiated
2	Coating test substance, irradiation
3	No test substance, no irradiation

4	Uncoated, irradiated

表 2 皮肤刺激反应评分

皮肤反应	积 分
红斑和焦痂形成	
无红斑	0
轻微红斑（勉强可见）	1
明显红斑	2
中度—重度红斑	3
严重红斑（紫红色）至轻微焦痂形成	4
水肿形成	
无水肿	0
轻微水肿（勉强可见）	1

皮肤反应	积 分
轻度水肿（皮肤隆起轮廓清楚）	2
中度水肿（皮肤隆起约 1mm）	3
重度水肿（皮肤隆起超过 1mm，范围扩大）	4
最高积分	8

Table 2 skin irritation response score

Skin reaction	Integral
Erythema and eschar formation	
No erythema	0
Slight erythema (barely visible)	1
Obvious erythema	2
Moderate to severe erythema	3
Severe erythema (purplish red) to slight eschar formation	4
Edema formation	
No edema	0
Slight edema (barely visible)	1

Skin reaction	Integral
Mild edema (clear contour of skin bulge)	2
Moderate oedema (skin swelling about 1mm)	3
Severe edema (skin swelling more than 1mm, expanded range)	4
Highest integral	8

6 结果评价

单纯涂受试物而未经照射区域未出现皮肤反应，而涂受试物后经照射的区域出现皮肤反应分值之和为 2 或 2 以上的动物数为 1 只或 1 只以上时，判为受试物具有光毒性。

6 Result evaluation

When the total number of animals with skin reaction score of 2 or more in the irradiated area is 1 or more, the test substance is deemed to have phototoxicity.

8 鼠伤寒沙门氏菌 / 回复突变试验

Salmonella Typhimurium / Reverse Mutation Assay

1 范围

本规范确定了鼠伤寒沙门氏菌 / 回复突变试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品的基因突变检测。

8 Salmonella Typhimurium / Reverse Mutation Assay

1 Range

This specification defines the basic principles, requirements and methods of Salmonella typhimurium / reverse mutation test.This specification is applicable to gene mutation detection of cosmetic raw materials and products.

2 定义

2.1 回复突变 reverse mutation

细菌在化学致突变物作用下由营养缺陷型回变到原养型(prototroph)。

2.2 基因突变 gene mutation

在化学致突变物作用下细胞 DNA 中碱基对的排列顺序发生变化。

2.3 碱基置换突变 base substitution mutation

引起 DNA 链上一个或几个碱基对的置换。

碱基置换有转换(transition)和颠换(transversion)两种形式。

转换是 DNA 链上的一个嘧啶被另一嘧啶所替代，或一个嘌呤被另一嘌呤所代替。颠换是 DNA 链上的一个嘧啶被另一嘌呤所替代，或一个嘌呤被另一嘧啶所代替。

2.4 移码突变 frameshift mutation

引起 DNA 链上增加或缺失一个或多个碱基对。

2.5 鼠伤寒沙门氏菌/回复突变试验 salmonella typhimurium/reverse mutation assay

利用一组鼠伤寒沙门氏组氨酸缺陷型试验菌株测定引起沙门氏菌碱基置换或移码突变的化学物质所诱发的组氨酸缺陷型(his-)→原养型(his+)回复突变的试验方法。

2.6 S9

经多氯联苯(PCB 混合物)或苯巴比妥钠和 β-萘黄酮结合诱导的大鼠制备肝匀浆，在 9000g 下离心 10min 后的肝匀浆上清液。

2 Definition

2.1 Reverse mutation

Under the action of chemical mutagens, bacteria changed from deficient to prototroph.

2.2 Gene mutation

Under the action of chemical mutagens, the sequence of base pairs in cell DNA changes.

2.3 Base substitution mutation

Causes the replacement of one or more base pairs on a DNA strand.

There are two forms of base substitution: transition and transversion.

Transformation is the replacement of one pyrimidine in the DNA chain by another pyrimidine, or the replacement of one purine by another purine.

Transversion is the substitution of one pyrimidine in a DNA strand by another purine, or one purine by another pyrimidine.

2.4 Frameshift mutation

Causes the addition or deletion of one or more base pairs on the DNA strand.

2.5 Salmonella typhimurium / reverse mutation assay

A group of Salmonella typhimurium histidine deficient test strains are used to determine the test method of histidine deficient (his -) prototrophic (his) + revertant mutation induced by chemicals that cause base replacement or frameshift mutation of Salmonella.

2.6 S9

The liver homogenate is prepared by the combination of PCBs or sodium phenobarbital with β - naphthalene flavone

The supernatant of liver homogenate is centrifuged at 9000g for 10min.

3 原理

鼠伤寒沙门氏组氨酸营养缺陷型菌株不能合成组氨酸，故在缺乏组氨酸的培养基上，仅少数自发回复突变的细菌生长。假如有致突变物存在，则营养缺陷型的细菌回复突变成原养型，因而能生长形成菌落，据此判断受试物是否为致突变物。

某些致突变物需要代谢活化后才能引起回复突变，故需加入经诱导剂诱导的大鼠肝制备的 S9 混合液。

4 仪器和设备

培养箱、恒温水浴、振荡水浴摇床、压力蒸汽消毒器、干热烤箱、低温冰箱(-80℃)或液氮生物容器、普通冰箱、天平(精密度 0.1g 和 0.0001g)、混匀振荡器、匀浆器、菌落计数器、低温高速离心机，玻璃器皿等。

3 Principle

The histidine deficient strains of Salmonella typhimurium could not synthesize histidine, so only a few bacteria with spontaneous reverse mutation grew on the medium lacking histidine. If there is a mutagen, the bacteria of the nutritional deficiency type will revert to the prototrophic type, so that they can grow and form colonies, so as to judge whether the test substance is a mutagen.

Some mutagens need to be metabolized and activated to cause reverse mutation, so it is necessary to add S9 mixture prepared by rat liver induced by inducer.

4 Instruments and equipment

Incubator, constant temperature water bath, oscillating water bath shaker, pressure steam sterilizer, dry heat oven, low temperature refrigerator (- 80 ℃) or liquid nitrogen biological container, general refrigerator, balance (precision 0.1g and 0.0001g), mixing oscillator, homogenizer, colony counter, low temperature high-speed centrifugal machine, glassware, etc.

5 培养基和试剂

5.1 0.5mmol/L 组氨酸-0.5mmol/L 生物素溶液

成分： L-组氨酸(MW 155) 78mg

D-生物素(MW 244) 122mg

加蒸馏水至 1000mL

配制：将上述成分加热，以溶解生物素，然后在 0.068MPa 下高压灭菌 20min。贮于 4℃冰箱。

5.2 顶层琼脂培养基

成分： 琼脂粉 1.2g

氯化钠 1.0g

加蒸馏水至 200mL

配制：上述成分混合后，于 0.103MPa 下高压灭菌 30min。实验时，加入 0.5mmol/L 组氨酸—0.5mmol/L 生物素溶液 20mL。

5 Media and reagents

5.1 0.5mmol/l histidine-0.5mmol/l biotin solution

Ingredient: L-histidine (MW 155): 78mg

D-biotin (MW 244): 122mg

Add distilled water to: 1000mL

Preparation: The above ingredients are heated to dissolve biotin, and then autoclaved at 0.068MPa for 20min minutes. Store in 4℃ refrigerator.

5.2 Top agar medium

Ingredients: agar powder 1.2g

Sodium chloride 1.0g

Add distilled water to 200ml

Preparation: after mixing the above ingredients, autoclave at 0.103mpa for 30min.In the experiment, 20ml of 0.5mmol/l histidine-0.5mmol/l biotin solution is added.

5.3 Vogel-Bonner (V-B) 培养基 E

成分：

枸橼酸(C6H8O7·H2O) 100g

磷酸氢二钾(K2HPO4) 500g

磷酸氢铵钠(NaNH4HPO4·4H2O) 175g

硫酸镁(MgSO4·7H2O) 10g

加蒸馏水至 1000mL

配制：先将前三种成分加热溶解后，再将溶解的硫酸镁缓缓倒入容量瓶中，加蒸馏水至 1000mL。于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.4 20%葡萄糖溶液

成分：葡萄糖 200g

加蒸馏水至 1000mL

配制：加少量蒸馏水加温溶解葡萄糖，再加蒸馏水至 1000mL。于 0.068MPa 下高压灭菌 20min。储于 4℃冰箱。

5.3 Vogel Bonner (V-B) medium e

Ingredient:

citric acid (C6H8O7 · H2O) 100g

Potassium hydrogen phosphate (K2HPO4) 500g
Sodium hydrogen phosphate (nanh4hpo4 · 4H2O) 175g
Magnesium sulfate (MgSO4 · 7H2O) 10g
Add distilled water to 1000ml

Preparation: first heat and dissolve the first three ingredients, then slowly pour the dissolved magnesium sulfate into the volumetric flask, add distilled water to 1000ml.Autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.4 20% glucose solution

Ingredient: glucose 200g
Add distilled water to 1000ml

Preparation: add a small amount of distilled water, warm and dissolve glucose, and then add distilled water to 1000ml.Autoclave at 0.068mpa 20min. Store in 4 ℃ refrigerator.

5.5 底层琼脂培养基

成分： 琼脂粉 7.5g
蒸馏水 480mL
V-B 培养基 E 10mL
20%葡萄糖溶液 10mL

配制：首先将前两种成分于 0.103MPa 下高压灭菌 30min 后，再加入后两种成分，充分混匀倒底层平板。按每皿 25mL 制备平板，冷凝固化后倒置于 37℃培养箱中 24h，备用。

5.6 营养肉汤培养基

成分： 牛肉膏 2.5g
胰 胨 5.0g
磷酸氢二钾(K2HPO4) 1.0g
加蒸馏水至 500mL

配制：将上述成分混合后，于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.5 Basal agar medium

Ingredients: agar powder 7.5G
Distilled water 480ml
V-B medium e 10ml
20% glucose solution 10ml

Preparation: first sterilize the first two ingredients under high pressure at 0.103mpa for 30min, then add the last two ingredients, mix them well and pour them into the bottom plate.Prepare the plate according to 25ml of each dish, after condensation and solidification, invert it into a 37 ℃ incubator for 24h, for standby.

5.6 Nutrient broth

Ingredients: beef paste 2.5G
Tryptone 5.0g
Dipotassium hydrogen phosphate (K2HPO4) 1.0g
Add distilled water to 500ml

Preparation: after mixing the above ingredients, autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.7 盐溶液(1.65mol/L KCl+0.4mol/L MgCl2)

成分： 氯化钾(KCl) 61.5g

氯化镁(MgCl2·6H2O) 40.7g

加蒸馏水至 500mL

配制：在水中溶解上述成分后，于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.8 0.2mol/L 磷酸盐缓冲液(pH7.4)

成分： 磷酸二氢钠(NaH2PO4·2H2O) 2.965g

磷酸氢二钠(Na2HPO4·12H2O) 29.015g

加蒸馏水至 500mL

配制：溶解上述成分后，于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.9 S9 混合液

成分 每毫升 S9 混合液

肝 S9 100ml

盐溶液 20ml

灭菌蒸馏水 380ml

0.2mol/L 磷酸盐缓冲液 500ml

辅酶 II(NADP) 4mmol

6-磷酸葡萄糖(G-6-P) 5mmol

配制：将辅酶 II 和 6-磷酸葡萄糖置于灭菌三角瓶内称重，然后按上述相反的次序加入各种成分， 使肝 S9 加到已有缓冲液的溶液中。该混合液必须临用现配，并保存于冰水浴中。实验结束，剩余 S9 混合液应该丢弃。

5.7 Salt solution (1.65mol/l KCl + 0.4mol/l MgCl2)

Ingredient: potassium chloride (KCl) 61.5g

Magnesium chloride (MgCl2 · 6H2O) 40.7g

Add distilled water to 500ml

Preparation: after dissolving the above ingredients in water, autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.8 0.2 mol/l phosphate buffer (pH7.4)

Ingredient: sodium dihydrogen phosphate (NaH2PO4 · 2H2O) 2.965g

Disodium hydrogen phosphate (Na2HPO4 · 12H2O) 29.015g

Add distilled water to 500ml

Preparation: after dissolving the above ingredients, autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.9 S9 mixture

Ingredients S9 mixture / ml

Liver S9 100ml

Salt solution 20ml

Sterilized distilled water 380ml

0.2mol/l phosphate buffer 500ml

Coenzyme II (NADP) 4mmol

6-Glucose phosphate (g-6-p) 5mmol

Preparation: put coenzyme II and glucose-6-phosphate into a sterilized triangular flask and weigh them, then add various components in the reverse order, so that liver S9 is added to the solution of the existing buffer solution. The mixture must be prepared temporarily and stored in an ice water bath. At the end of the experiment, the remaining S9 mixture should be discarded.

5.10 菌株鉴定用和特殊用途试剂

5.10.1 组氨酸—生物素平板

成分：琼脂粉 15g

蒸馏水 944mL

(V-B)培养基 E 20mL

20%葡萄糖 20mL

灭菌盐酸组氨酸水溶液(0.5g/100mL) 10mL

灭菌 0.5mmol/L 生物素溶液 6mL

配制：高压灭菌琼脂和水后，将灭菌 20%葡萄糖，V-B 培养基和组氨酸溶液加进热的琼脂溶液中。待溶液稍为冷却后，加入灭菌生物素，混匀，浇制平板。

5.10.2 氨苄青霉素平板和氨苄青霉素/四环素平板

成分：琼脂粉 15g

蒸馏水 940mL

(V-B)盐溶液 20mL

20%葡萄糖 20mL

灭菌盐酸组氨酸溶液（0.5g/100mL） 10mL

灭菌 0.5mmol/L 生物素溶液 6mL

氨苄青霉素溶液(8mg/mL 于 0.02mol/LNaOH 中) 3.15mL

四环素溶液(8mg/mL 于 0.02mol/L HCl 中) 0.25mL

配制：琼脂和水高压灭菌 20min，将无菌的葡萄糖、VB 盐溶液和组氨酸—生物素溶液加进热的溶液中去，混匀。冷却至大约 50℃，无菌条件下加入四环素溶液和/或氨苄青霉素溶液。

应该在倾注琼脂平板后几天内，制备主平板。

5.10.3 营养琼脂平板

成份：琼脂粉 7.5g

营养肉汤培养基 500mL

配制：于 0.103MPa 下高压灭菌 30min 后倾注平板。

5.10 Reagents for strain identification and special use

5.10.1 Histidine biotin plate

Ingredients: agar powder 15g

Distilled water 944ml

(V-B) medium e 20ml

20% glucose 20ml

Sterilized histidine hydrochloride aqueous solution (0.5g / 100ml) 10ml

Sterilization 0.5mmol/l biotin solution 6ml

Preparation: after high-pressure sterilization of agar and water, sterilized 20% glucose, V-B medium and histidine solution are added into hot agar solution.After the solution is slightly cooled, add the sterilized biotin, mix well, and pour the plate.

5.10.2 Ampicillin plate and ampicillin / tetracycline plate

Ingredients: agar powder 15g

Distilled water 940ml

(V-B) salt solution 20ml

20% glucose 20ml

Sterilization histidine hydrochloride solution (0.5g / 100ml) 10ml

Sterilization 0.5mmol/l biotin solution 6ml

Ampicillin solution (8mg / ml in 0.02mol/lnaoh) 3.15ml

Tetracycline solution (8mg / ml in 0.02mol/l HCl) 0.25ml

Preparation: sterilize agar and water under high pressure for 20min, add sterile glucose, VB salt solution and histidine biotin solution into hot solution, mix well.Cool to about 50 ℃, and add tetracycline solution and / or ampicillin solution in sterile condition.

The main plate should be prepared within a few days of pouring the agar plate.

5.10.3 Nutrient agar plate

Ingredients: agar powder 7.5G

Nutrient broth medium 500ml

Preparation: pour the plate after 30 minutes of high-pressure sterilization at 0.103mpa.

6 试验菌株及其 Th 物学特性鉴定

6.1 试验菌株

采用 TA97、TA98、TA100 和 TA102 一组标准测试菌株。

6.2 生物学特性鉴定

新获得的或长期保存的菌种，在试验前必须进行菌株的生物特性鉴定。菌株鉴定的判断标准，如表 1 所示。

6 Test strains and identification of Th physical properties

6.1 Test strain

Ta97, TA98, TA100 and TA102 are used to test the strains.

6.2 Identification of biological characteristics

For the newly obtained or long-term preserved strains, the biological characteristics of the strains must be identified before the test. The judgment criteria for strain identification are shown in Table 1.

表 1 试验菌株鉴定的判断标准

菌株	组氨酸缺陷	脂多糖屏障缺损	氨苄青霉素抗性	切除修复缺损	四环素抗性	自发回变菌落数*
TA97	+	+	+	+	-	90—180
TA98	+	+	+	+	-	30—50
TA100	+	+	+	+	-	100—200
TA102	+	+	+	-	+	240—320
注	“+”表示需要组氨酸	“+”表示具有 rfa 突变	“+”表示具有 R 因子	“+”表示具有△uvrB 突变	“+”表示具有 pAQ1 质粒	* 在体外代谢活化条件下自发回变菌落数略增

Table 1 criteria for identification of test strains

Strain	Histidine deficiency	Lipopolysaccharide barrier defect	Ampicillin resistance	Excision and repair of defect	tetracycline resistance	Spontaneous reversion colony number*
TA97	+	+	+	+	-	90—180
TA98	+	+	+	+	-	30—50
TA100	+	+	+	+	-	100—200
TA102	+	+	+	-	+	240—320
notes	"+" indicates the need for histidine	"+" indicates RFA mutation	"+" means having R factor	"+" indicates a mutation of △uvrb	"+" indicates the presence of paq1 plasmid	*Under the condition of metabolic activation in vitro, the number of self returning colonies increased slightly

6.2.1 组氨酸缺陷

原理：组氨酸缺陷型试验菌株本身不能合成组氨酸，只能在补充组氨酸的培养基上生长，而在缺乏组氨酸的培养基上，则不能生长。

鉴定方法：将测试菌株增菌液分别于含组氨酸培养基平板和无组氨酸平板上划线，于

37℃下培养 24h 后观察结果。

结果判断：组氨酸缺陷型菌株在含组氨酸平板上生长，而在无组氨酸平板上则不能生长。

6.2.1 Histidine deficiency

Principle: histidine deficient test strains can't synthesize histidine by themselves, and can only grow on the medium supplemented with histidine, but can't grow on the medium lacking histidine.

Identification method: mark the enrichment solution of test strain on the plate containing histidine and the plate without histidine, respectively

The results are observed 24 hours after incubation at 37℃.

The results showed that the histidine deficient strain could not grow on the histidine containing plate, but on the histidine free plate.

6.2.2 脂多糖屏障缺损

原理：具有深粗糙（rfa）的菌株，其表面一层脂多糖屏障缺损，因此一些大分子物质如结晶紫能穿透菌膜进入菌体，从而抑制其生长，而野生型菌株则不受其影响。

鉴定方法：吸取待测菌株增菌液 0.1mL 于营养琼脂平板上划线，然后将浸湿的0.1%结晶紫溶液滤纸条与划线处交叉放置。37℃下培养 24h 后观察结果。

结果判断：假若待测菌在滤纸条与划线交叉处出现一透明菌带，说明该待测菌株具有 rfa 突变。

6.2.2 Lipopolysaccharide barrier defect

Principle: for strains with deep roughness (RFA), there is a layer of lipopolysaccharide barrier defect on the surface. Therefore, some macromolecular substances such as crystal violet can penetrate the bacterial membrane and enter into the bacteria, thus inhibiting its growth, while wild-type strains are not affected by it.

Identification method: draw 0.1ml of enrichment solution of the strain to be tested and scribe it on the nutrient agar plate, then place the filter paper strip of 0.1% crystal violet solution soaked in water across the scribe. The results are observed at 37 ℃ for 24 hours.

Results Judgment: If a transparent bacterial band appears at the intersection of the filter paper strip and the scribe line, the strain to be tested has rfa mutation.

6.2.3 氨苄青霉素抗性

原理：含 R 因子的试验菌株对氨苄青霉素有抗性。因为 R 因子不太稳定，容易丢失，故用氨苄青霉素确定该质粒存在与否。

鉴定方法：吸取待测菌株增菌液 0.1mL，在氨苄青霉素平板上划线，37℃下培养 24h 后观察结果。

结果判断；假若测试菌在氨苄青霉素平板上生长，说明该测试菌具有抗氨苄青霉素作用，表示含 R 因子，否则，表示测试菌不含 R 因子或 R 因子丢失。

6.2.4 紫外线敏感性

原理：具有△uvrB 突变的菌株对紫外线敏感，当受到紫外线照射后，不能生长，而具有野生型切除修复酶的菌株，则能照常生长。

鉴定方法：吸取待测菌株增菌液 0.1mL 于营养琼脂平板上划线，用黑纸盖住平板的一半，置紫外灯下照射（15W，距离 33cm）8 秒钟。置 37℃下孵育 24h 后观察结果。

结果判断：具有△uvrB 突变的菌株对紫外线敏感，经辐射后细菌不生长，而具有完整的切除修复系统的菌株，则照常生长。

6.2.3 Ampicillin resistance

Principle: the test strain containing R factor is resistant to ampicillin. Because R factor is not stable and easy to be lost, ampicillin is used to determine the existence of the plasmid.

Identification method: take 0.1ml of enrichment solution of the strain to be tested, draw lines on the ampicillin plate, and culture at 37 ℃ for 24h, then observe the results.

Results: if the test bacteria grow on the ampicillin plate, it indicates that the test bacteria has the effect of anti-ampicillin, indicating that it contains R factor, otherwise, it indicates that the test bacteria does not contain R factor or R factor is lost.

6.2.4 UV sensitivity

Principle: the strain with △ uvrB mutation is sensitive to UV, and can't grow when it is irradiated by UV, while the strain with wild type excision and repair enzyme can grow as usual.

Identification method: draw 0.1ml of enrichment solution of the strain to be tested, scribe on the nutrient agar plate, cover half of the plate with black paper, and irradiate under the ultraviolet lamp (15W, distance 33cm) for 8 seconds. The results are observed after incubation at 37 ℃ for 24 hours.

The results showed that the strains with △ uvrB mutation are sensitive to UV, and the bacteria did not grow after irradiation, but the strains with complete removal and repair system grew as usual.

6.2.5 四环素抗性

原理：具有 pAQI 的菌株对四环素有抗性。

鉴定方法：吸取待测菌株增菌液 0.1mL 于氨苄青霉素/四环素平板上划线，置 37℃下孵育 24h 后观察结果。

结果判断：假若测试菌照常在氨苄青霉素/四环素平板上生长，表明该测试菌株对氨苄青霉素和四环素两者有抗性，具有 pAQI 质粒，否则，说明测试菌株不含 pAQI 质粒。

6.2.6 自发回变

原理：每种试验菌株都以一定的频率自发地产生回变，称为自发回变。这种自发回变是每种试验菌株的一项特性。

鉴定方法：将待测菌株增菌液 0.1mL 加到 2mL 含组氨酸—生物素的顶层琼脂培养基的试管内，混匀后铺到于底层琼脂平板上，待琼脂固化后，置 37℃培养箱中孵育 48h 后记数每皿回变菌落数。

结果判断：每种标准测试菌株的自发回变菌落数应符合表 1 要求。经体外代谢活化后的自发回变菌落数，要比直接作用下的略高。

6.2.5 tetracycline resistance

Principle: strains with pAQI are resistant to tetracycline.

Identification method: take 0.1ml of enrichment solution of the strain to be tested, draw a line on the ampicillin / tetracycline plate, incubate at 37 ℃ for 24h, and observe the results.

The results showed that if the test strain grew on the ampicillin / tetracycline plate as usual, the test strain is resistant to both ampicillin and tetracycline, and had pAQI plasmid, otherwise, the test strain did not contain pAQI plasmid.

6.2.6 Spontaneous reversion

Principle: each test strain produces spontaneous reversion at a certain frequency, which is called spontaneous reversion. This spontaneous reversion is a characteristic of each test strain.

Identification method: add 0.1ml of enrichment solution to 2ml of top agar medium containing histidine biotin, mix well and spread it on the bottom agar plate. After the agar solidifies, incubate in 37 ℃ incubator for 48h and count the number of changed colonies in each dish.

Results judgment: the number of spontaneous revertant colonies of each standard test strain should meet the requirements of Table 1.The number of spontaneous revertant colonies after in vitro metabolism activation is slightly higher than that under direct action.

6.2.7 回变特性—诊断性试验

原理：每种试验菌株对诊断性诱变剂回变作用的性质以及 S9 混合液的效应不一。

鉴定方法：按照平板掺入试验的操作步骤进行。将受试物换成诊断性诱变剂。

结果判断：标准菌株对某些诊断性诱变剂特有的回变结果参见表 2。

6.2.7 Return characteristic diagnostic test

Principle: the nature of each test strain's reversion to diagnostic mutagen and the effect of S9 mixture are different.

Identification method: according to the operation steps of plate mixing test. Replace the test substance with diagnostic mutagen.

Results judgment: see Table 2 for the specific reversion results of standard strains to some diagnostic mutagens.

表 2 测试菌株的回变性

诱变剂	剂量(mg)	S9	TA97	TA98	TA100	TA102

柔毛霉素	6.0	-	124	3123	47	592
叠氮化钠	1.5	-	76	3	3000	188
ICR—191	1.0	-	1640	63	185	0
链霉黑素	0.25	-	inh	inh	inh	2230
丝裂霉素 C	0.5	-	inh	inh	inh	2772
2,4,7-三硝基-9-芴酮	0.20	-	8377	8244	400	16
4-硝基-O-次苯二胺	20	-	2160	1599	798	0
4-硝基喹啉-N-氧化物	0.5	-	528	292	4220	287
甲基磺酸甲酯	1.0	-	174	23	2730	6586
2-氨基芴	10	+	1742	6194	3026	261
苯并（a）芘	1.0	+	337	143	937	255

inh 表示抑菌。表中数值均已扣除溶剂对照回变菌落数。

Table 2. Reversion of tested strains

Mutagen	Dose (mg)	S9	TA97	TA98	TA100	TA102	
Pilomycin	6.0	-	124	3123	47	592	
sodium azide	1.5	-	76	3	3000	188	
ICR-191	1.0	-	1640	63	185		0
Streptomycin melanin	0.25	-	inh	inh	inh	2230	
Mitomycin C	0.5	-	inh	inh	inh	2772	
2,4,7-Trinitro-9-fluorenone	0.20	-	8377	8244	400	16	
4-Nitro-o-phenylenediamine	20	-	2160	1599	798		0
4-Nitroquinoline-n-oxide	0.5	-	528	292	4220	287	
methyl sulfonate	1.0	-	174	23	2730	6586	
2-Aminofluorene	10	+	1742	6194	3026	261	
Benzo (a) pyrene	1.0	+	337	143	937	255	

INH indicates bacteriostasis. The number of revertant colonies of solvent control has been deducted from the values in the table.

7 大鼠肝微粒体酶的诱导和 S9 的制备

7.1 诱导

选择健康雄性成年大鼠，体重 200g 左右。将多氯联苯（PCB 混合物）溶于玉米油中，浓度为 200mg/mL，按 500mg/kg 体重一次腹腔注射，5d 后处死动物，处死前禁食 12h。

也可采用苯巴比妥钠和 β-萘黄酮联合诱导的方法进行制备。经口或腹腔注射给予 80mg/kg 苯巴比妥钠和 80mg/kgβ-萘黄酮，连续 3 天，处死前禁食 16h。

7 Induction of rat liver microsomal enzyme and preparation of S9

7.1 Induce

Healthy male adult rats are selected with a body weight of about 200g.The polychlorinated biphenyls (PCB mixture) are dissolved in corn oil at a concentration of 200mg / ml, and injected intraperitoneally once at a weight of 500mg / kg. The animals are killed five days later and fasted for 12 hours before the death.

It can also be prepared by the combined induction method of phenobarbital sodium and beta-naphthoflavone. 80mg/kg phenobarbital sodium and 80mg/kgβ- naphthoflavone were given orally or intraperitoneally for 3 consecutive days and fasting for 16 hours before execution.

7.2 S9 制备

首先，用 75%酒精消毒动物皮毛，剖开腹部。在无菌条件下，取出肝脏，去除肝脏的结缔组织，用冰浴的 0.15mol/L 氯化钾溶液淋洗肝脏，放入盛有 0.15mol/L 氯化钾溶液的烧杯里。按每克肝脏加入 0.15mol/L 氯化钾溶液 3mL。用电动匀浆器制成肝匀浆，再在低温高速离心机上，在 4℃条件下，以 9000g 离心 10min，取其上清液（S9）分装于塑料管中。每管装 2 mL —3 mL。储存于液氮生物容器中或-80℃冰箱中备用。

上述全部操作均在冰水浴中和无菌条件下进行。制备肝 S9 所用一切手术器械、器皿等，均经灭菌消毒。S9 制备后，其活力需经诊断性诱变剂进行鉴定。

7.2 S9 preparation

First, disinfect animal fur with 75% alcohol and cut open the abdomen. Under sterile condition, take out the liver, remove the connective tissue of the liver, wash the liver with 0.15mol/l potassium chloride solution of ice bath, and put it into a beaker containing 0.15mol/l potassium chloride solution. Add 3ml of 0.15mol/l potassium chloride solution per gram of liver. The liver homogenate is made by electric homogenizer, and then centrifuged at 4 ℃ for 10 minutes at 9000g on a low-temperature high-speed centrifuge. The supernatant (S9) is separated into plastic tubes.2-3 ml for each tube. Store in liquid nitrogen biological container or refrigerator at - 80 ℃ for standby.

All the above operations are carried out in ice water bath and sterile condition. All surgical instruments and utensils used in the preparation of liver S9 are sterilized. After S9 preparation, its activity needs to be identified by diagnostic mutagen.

8 溶剂的选择

如果受试物为水溶性，可用灭菌蒸馏水作为溶剂；如为脂溶性，应选择对试验菌株毒性低且无致突变性的有机溶剂，常用的有二甲基亚砜（DMSO）、丙酮、95%乙醇。一般操作中，为了减少误差和溶剂的影响，常按每皿使用剂量用同一溶剂配成不同的浓度，固定加入量为 100ml。

8 Solvent selection

If the test substance is water-soluble, sterilized distilled water can be used as the solvent; if it is fat soluble, the organic solvent with low toxicity to the test strain and no mutagenicity should be selected, commonly used are DMSO, acetone and 95% ethanol. In general operation, in order to reduce the error and the influence of solvent, the same solvent is often used to prepare different concentrations according to the dosage of each dish, and the fixed dosage is 100ml.

9 剂量的设计

决定受试物最高剂量的标准是对细菌的毒性及其溶解度。自发回变数的减少，背景菌变得清晰或被处理的培养物细菌存活数减少，都是毒性的标志。

对原料而言，一般最高剂量组可为 5mg/皿或 5ul/皿。对产品而言，有杀菌作用的受试物，最高剂量可为最低抑菌浓度，无杀菌作用的受试物，最高剂量可为原液。受试物至少应设四个剂量组。每个剂量均做三个平行平板。

9 Dose design

The standard for determining the maximum dose of test substance is its toxicity to bacteria and its solubility. The decrease of spontaneous return variable, the clear background bacteria or the decrease of bacterial survival in the treated culture are all signs of toxicity.

For raw materials, the general maximum dose group can be 5mg / dish or 5ul / dish. For the product, the highest dose of the test substance with bactericidal effect can be the lowest bacteriostatic concentration, the highest dose of the test substance without bactericidal effect can be the original solution. There should be at least four dose groups for the test substance. Three parallel plates are made for each dose.

10 试验操作步骤

10.1 增菌培养

取营养肉汤培养基 5mL，加入无菌试管中，将主平板或冷冻保存的菌株培养物接种于营养肉汤培养基内，37℃振荡（100 次/min）培养 10h。该菌株培养物应每毫升不少于 1—2×109 活菌数。

10.2 平板掺入法

实验时，将含 0.5mmol/L 组氨酸-0.5mmol/L 生物素溶液的顶层琼脂培养基 2.0mL 分装于试管中，45℃水浴中保温，然后每管依次加入试验菌株增菌液 0.1mL，受试物溶液 0.1mL 和 S9 混合液 0.5mL（需代谢活化时），充分混匀，迅速倾入底层琼脂平板上，转动平板，使之分布均匀。水平放置待冷凝固化后，倒置于 37℃培养箱里孵育 48h。记数每皿回变菌落数。

实验中，除设受试物各剂量组外，还应同时设空白对照、溶剂对照、阳性诱变剂对照和无菌对照。

10 Test operation steps

10.1 Enrichment culture

Take 5ml of nutrient broth culture medium, add it into sterile test tube, inoculate the main plate or frozen preserved strain culture into nutrient broth culture medium, and

culture for 10h under 37 ℃ shaking (100 times / min).The number of viable strains should be no less than 1-2 × 109 per ml.

10.2 Plate incorporation method

In the experiment, the top layer agar medium containing 0.5mmol/l histidine-0.5mmol/l biotin solution is divided into 2.0ml tubes, which are kept warm in a 45 ℃ water bath, and then 0.1ml of enrichment solution, 0.1ml of test solution and S9 mixture are added to each tube in turn0.5ml (when metabolic activation is required), mix it well, pour it into the bottom agar plate quickly, and rotate the plate to make it evenly distributed. After condensation and solidification, it is placed horizontally and incubated in 37 ℃ incubator for 48h.Count the number of changed colonies per dish.

In the experiment, in addition to each dose group of test substance, blank control, solvent control, positive mutagen control and sterile control should also be set.

11 数据处理和结果判断

记录受试物各剂量组、空白对照（自发回变）、溶剂对照以及阳性诱变剂对照的每皿回变菌落数，并求平均值和标准差。

如果受试物的回变菌落数是溶剂对照回变菌落数的两倍或两倍以上，并呈剂量-反应关系者，则该受试物判定为致突变阳性；受试物在任何一个剂量条件下，出现阳性反应并有可重复性，则该受试物判定为致突变阳性。

受试物经上述四个试验菌株测定后，只要有一个试验菌株，无论在加 S9 或未加 S9 条件下为阳性，均可报告该受试物对鼠伤寒沙门氏菌为致突变阳性。

如果受试物经四个试验菌株检测后，无论加 S9 和未加 S9 均为阴性，则可报告该受试物为致突变阴性。

11 Data processing and result judgment

The colony number of each dish of each dose group, blank control (spontaneous reversion), solvent control and positive mutagen control are recorded, and the mean value and standard deviation are calculated.

If the number of revertant colonies of the test substance is twice or more than that of the solvent control, and there is a dose-response relationship, the test substance is determined to be mutagenic positive; if the test substance has a positive reaction and repeatability under any dose condition, the test substance is determined to be mutagenic positive.

After the test substance is tested by the above four test strains, as long as one test strain is positive with or without S9, the test substance can be reported to be mutagenic positive for salmonella typhimurium.

If the test substance is negative with or without S9 after being tested by four test strains, the test substance can be reported as mutagenic negative.

9 体外哺乳动物细胞染色体畸变试验

1 范围

本规范规定了体外哺乳动物细胞染色体畸变试验的基本原则、要求和方法。本规范适用于检测化妆品原料及其产品的致突变性。

2 试验目的

本试验是用于检测培养的哺乳动物细胞染色体畸变，以评价受试物致突变的可能性。

9 In Vitro Mammalian Cells Chromosome Aberration Test

1 Range

This specification specifies the basic principles, requirements and methods of chromosome aberration test of mammalian cells in vitro. This specification is applicable to the detection of mutagenicity of cosmetic raw materials and products.

2 Test purpose

The purpose of this study is to detect chromosome aberrations in cultured mammalian cells and to evaluate the possibility of mutagenicity of the test substance.

3 定义

3.1 结构畸变 structural aberration

在细胞分裂的中期相阶段，用显微镜检出的染色体结构改变，表现为缺失、断片、互换等。结构畸变可分为以下两类。

3.1.1 染色体型畸变 chromosome-type aberration

染色体结构损伤，表现为在两个染色单体相同位点均出现断裂或断裂重组的改变。

3.1.2 染色单体型畸变 chromatid-type aberration

染色体结构损伤，表现为染色单体断裂或染色单体断裂重组的损伤。

3.2 有丝分裂指数 mitotic index

中期相细胞数与所观察的细胞总数之比值；是一项反映细胞增殖程度的指标。

3 Definition

3.1 Structural aberration

In the metaphase of cell division, the chromosome structural changes detected by microscope are deletion, fragment, exchange, etc. Structural aberration can be divided into the following two categories.

3.1.1 Chromosome-type aberration

The damage of chromosome structure shows the change of breakage or recombination at the same site of two chromatids.

3.1.2 chromatid-type aberration

The damage of chromosome structure is the damage of chromatid breaking or chromatid breaking and recombination.

3.2 Mitotic index

The ratio of the number of metaphase cells to the total number of observed cells is an index reflecting the degree of cell proliferation.

4 试验基本原则

在加入和不加入代谢活化系统的条件下，使培养的哺乳动物细胞暴露于受试物中。用中期分裂相阻断剂（如秋水仙素或秋水仙胺）处理，使细胞停止在中期分裂相，随后收获细胞，制片，染色，分析染色体畸变。

大部分的致突变剂导致染色单体型畸变，偶有染色体型畸变发生。虽然多倍体的增加可能预示着有染色体数目畸变的可能，但本方法并不适合用于测定染色体的数目畸变。

4 Basic principles of test

The cultured mammalian cells are exposed to the test substance with or without metabolic activation system. The metaphase blocker (such as colchicine or colchicine) is used to stop the cell from metaphase, and then the cell is harvested, sectioned, stained and analyzed for chromosomal aberrations.

Most of the mutagens lead to chromosomal aberrations, with occasional chromosomal aberrations. Although the increase of polyploidy may indicate the possibility of chromosome number aberrations, this method is not suitable for the determination of chromosome number aberrations.

5 试验方法

5.1 试剂和受试物制备

5.1.1 阳性对照物：可根据受试物的性质和结构选择适宜的阳性对照物，阳性对照物应是已知的断裂剂，能引起可检出的、并可重复的阳性结果。当外源性活化系统不存在时，可使用甲磺酸甲酯（methyl methanesulphonate (MMS)）、甲磺酸乙酯（ethylmethanesulphonate(EMS)）、乙基亚硝基脲(ethyl nitrosourea)、丝裂霉素

C(mitomycin C)、4-硝基喹啉-N-氧化物（4-nitroquinoline-N-oxide）。当外源性活化系统存在时，可使用苯并（a）芘[benzo(a)pyrene]、环磷酰胺(cyclophosphamide)。

5.1.2 阴性对照物：应设阴性对照，即仅含和受试物组相同的溶剂，不含受试物，其他处理和受试物组完全相同。此外，如未能证实所选溶剂不具有致突变性，溶剂对照与本实验室空白对照背景资料有明显差异，还应设空白对照。

5 test method

5.1 Reagent and test substance preparation

5.1.1 Positive control substance: A suitable positive control substance can be selected according to the nature and structure of the test substance. The positive control substance should be a known breaking agent, which can cause detectable and repeatable positive results. When the exogenous activation system does not exist, methyl methanesulphonate (MMS), ethylmethanesulphonate (EMS), ethyl nitrosourea, mitomycin C, 4- nitroquinoline -N- oxide can be used. When an exogenous activation system is present, benzo (a) pyrene [benzo(a)pyrene], cyclophosphamide can be used.

5.1.2 Negative control substance: negative control substance shall be set, that is, only containing the same solvent as the test substance group, excluding the test substance, and other treatments are exactly the same as the test substance group. In addition, if it is not confirmed that the selected solvent does not have mutagenicity, the background data of solvent control and blank control in our laboratory are significantly different, and blank control should be set up.

5.1.3 受试物

5.1.3.1 受试物的配制：固体受试物需溶解或悬浮于溶剂中，用前稀释至适合浓度；液体受试物可以直接加入试验系统和/或用前稀释至适合浓度。受试物应在使用前新鲜配制，否则就必须证实贮存不影响其稳定性。

5.1.3.2 溶剂的选择：溶剂必须是非致突变物，不与受试物发生化学反应，不影响细胞存活和 S9 活性。首选溶剂是培养液（不含血清）或水。二甲基亚砜（DMSO）也是常用溶剂，使用时浓度不应大于 0.5%。

5.1.3 Test substance

5.1.3.1 Preparation of test substance: the solid test substance shall be dissolved or suspended in the solvent and diluted to the appropriate concentration before use; the liquid test substance can be directly added into the test system and / or diluted to the appropriate concentration before use. The test substance shall be freshly prepared before use, otherwise it must be confirmed that storage does not affect its stability.

5.1.3.2 Selection of solvent: the solvent must be non mutagenic, not react with the test substance, not affect cell survival and S9 activity. The preferred solvent is culture medium (without serum) or water. Dimethyl sulfoxide (DMSO) is also a common solvent, the concentration should not be more than 0.5% when used.

5.1.3.3 受试物浓度设置

（1）最高浓度的选择：

决定最高浓度的因素是细胞毒性、受试物在试验系统中的溶解度以及 pH 或渗克分子浓度(osmolality)的改变。

（2）细胞毒性的确定：

应使用指示细胞完整性和生长情况的指标，在活化系统存在或不存在的两种条件下确定细胞毒性，例如细胞覆盖程度（degree of confluency）、存活细胞计数(viable cell counts)或有丝分裂指数(mitotic index)。应在预试验中确定细胞毒性和溶解度。

（3）剂量设置：

①至少应设置 3 个可供分析的浓度。当有细胞毒性时，其浓度范围应包括从最大毒性至几乎无毒性；通常浓度间隔系数不大于 2 —√10。

②在收获细胞时，最高浓度应能明显降低细胞覆盖程度、细胞计数或有丝分裂指数（均应大于 50%）。

③对于那些相对无细胞毒性的化合物，最高浓度应是 5μl/mL，5mg/mL 或 0.01mol/L。

④对于相对不溶解的物质，当浓度低于不溶解浓度时仍无毒性，则最高剂量应是，当处理期结束时，在最终培养液中溶解度限值以上的一个浓度。在某些情况下（即仅当高于最低不溶解浓度时才发生细胞毒性），应使用一个以上可看见沉淀的浓度。最好在试验处理开始和结束时均评价溶解度，因为由于细胞、S9 等的存在，在试验系统内在暴露过程中溶解度可能变化。不溶解性可用肉眼鉴别，但沉淀不能影响观察。

5.1.4 培养液：采用 MEM(Eagle)，并加入非必需氨基酸和抗菌素（青、链霉素，按 100IU/mL），胎牛血清或小牛血清按 10%加入。也可选用其他合适的培养液。

5.1.3.3 Test substance concentration setting

(1) Selection of the highest concentration:

The factors that determine the highest concentration are cytotoxicity, solubility of the test substance in the test system, and changes in pH or osmolality.

(2) Determination of cytotoxicity:

Cell toxicity should be determined under two conditions, with or without activation system, using indicators indicating cell integrity and growth, such as cell coverage, viable cell counts, or mitotic index. Cytotoxicity and solubility should be determined in the pre-test.

(3) Dose setting:

① At least 3 concentrations for analysis shall be set. When there is cytotoxicity, its concentration range should include from maximum toxicity to almost no toxicity; Usually the concentration interval coefficient is not more than 2-√ 10.

② When harvesting cells, the highest concentration should be able to significantly reduce cell coverage, cell count or mitotic index (all should be greater than 50%).

③ For those relatively non cytotoxic compounds, the maximum concentration should be 5 μ L / ml, 5mg / ml or 0.01mol/l.

④ For relatively insoluble substances, when the concentration is lower than the insoluble concentration, it is still non-toxic. The maximum dose should be a concentration above the solubility limit in the final culture solution at the end of the treatment period. In some cases (i.e. cytotoxicity occurs only when above the minimum insoluble concentration), more than one concentration of visible precipitates should be used. It is best to evaluate solubility at the beginning and end of the treatment because solubility may change during exposure within the test system due to the presence of

cells, S9, etc. The insolubility can be identified by naked eyes, but the precipitation can not affect the observation.

5.1.4 Culture medium: MEM (Eagle), non essential amino acids and antibiotics (penicillin and streptomycin, 100iu / ml) are added, and fetal bovine serum or calf serum is added at 10%.Other suitable culture medium can also be selected.

5.1.5 活化系统

通常使用的是 S9 混合物（S9 mix）。S9 是从经酶诱导剂（Aroclor 1254 或苯巴比妥钠和 β-萘黄酮联合使用）处理的啮齿动物肝脏获得的。S9 的制备同 Ames 试验。S9 的使用浓度为 1%—10%（终浓度）。S9 mix 中所加辅助因子的量由各实验室自行决定，但需对 S9 mix 的活性进行鉴定，必须能明显活化阳性对照物。也可使用下述

S9 0.125ml
MgC12 （0.4 mol/L） 0.02 ml
KC1（1.65mol/L） 0.02 ml
葡萄糖-6-磷酸 1.791mg
辅酶Ⅱ（氧化型，NADP） 3.0615mg
用无血清 MEM 培养液补足至 1mL.

5.1.5 Activation system

Usually S9 mix is used. S9 is obtained from rodent liver treated with enzyme inducer (Aroclor 1254 or sodium phenobarbital combined with β-naphthoflavone). The preparation of S9 was the same as Ames test. The concentration of S9 is 1%-10% (final concentration). The amount of auxiliary factors added in S9 mix is determined by each laboratory, but the activity of S9 mix must be identified and the positive control substance must be activated obviously. The following can also be used

S9 0.125ml
MgC12 （0.4 mol/L） 0.02 ml
KC1（1.65mol/L） 0.02 ml
Glucose-6-phosphate 1.791mg
Coenzyme Ⅱ (oxidation type, NADP) 3.0615mg
Make up to 1ml with serum-free MEM medium

5.2 试验步骤

5.2.1 细胞：可使用已建立的细胞株或细胞系，也可使用原代培养细胞。所使用的细胞应该在生长性能、染色体数目和核型、自发的染色体畸变率等方面有一定的稳定性。推荐使用中国地鼠卵巢（CHO）细胞株或中国地鼠肺（CHL）细胞株。

5.2.2 试验时，应同时设阳性对照物，阴性对照物和至少 3 个可供分析的受试物浓度组。

5.2.3 试验前一天，将一定数量的细胞接种于培养皿（瓶）中，放 CO2 培养箱内培养。

5.2.4 试验需在加入和不加入 S9 mix 的条件下进行。试验时，吸去培养皿（瓶）中的培养液，加入一定浓度的受试物、S9 mix（不加 S9 mix 时，需用培养液补足）以及一定量不含血清的培养液，放培养箱中处理 3h—6h。结束后，吸去含受试物的培养液，用 Hanks 液洗细胞 3 次，加入含 10%胎牛血清的培养液，放回培养箱，于 24h 内收获细胞。于收获前 2h—4h，加入细胞分裂中期阻断剂（如用秋水仙素，作用时间为 4h，终浓度为 1μg/mL）。

当受试物为原料时，如果在上述加入和不加入 S9 mix 的条件下均获得阴性结果，则尚需补加另外的试验，即在不加 S9 mix 的条件下，使受试物与试验系统的接触时间延长至 24h。

当难以得出明确结论时，应更换试验条件，如改变代谢活化条件、受试物与试验系统接触时间等重复试验。

5.2 Test procedure

5.2.1 Cell: the established cell line or cell line can be used, or the primary culture cell can be used. The cells used should have certain stability in growth performance, chromosome number and karyotype, spontaneous chromosome aberration rate, etc.I t is recommended to use Chinese hamster ovary (CHO) cell line or Chinese hamster lung (CHL) cell line.

5.2.2 During the test, positive control substance, negative control substance and at least three test substance concentration groups that can be analyzed shall be set at the same time.

5.2.3 On the day before the experiment, a certain number of cells are inoculated in a culture dish (bottle) and cultured in a CO2 incubator.

5.2.4 The test shall be conducted with and without S9 mix. During the test, the culture medium in the culture dish (bottle) is sucked away, and a certain concentration of test substance, S9 mix (if not, it needs to be supplemented with culture medium) and a certain amount of culture medium without serum are added, and then it is put into the incubator for 3h-6h.After that, the culture medium containing the test substance is sucked out, the cells are washed with Hanks solution three times, the culture medium containing 10% fetal bovine serum is added, and the cells are returned to the incubator and harvested within 24 hours. Two hours to four hours before harvest, add the medium-term blocker of cell division (such as colchicine, the action time is four hours, the final concentration is 1 μ g / ml).

When the test object is used as the raw material, if negative results are obtained under the above conditions with and without S9 mix, additional tests need to be added, i.e. the contact time between the test object and the test system is extended to 24h without S9 mix.

When it is difficult to reach a definite conclusion, the test conditions should be changed, such as changing metabolic activation conditions, contact time between the test object and the test system, etc.

5.2.5 收获细胞时，用 0.25%胰蛋白酶溶液消化细胞，待细胞脱落后，加入含 10%胎牛或小牛血清的培养液终止胰蛋白酶的作用，混匀，放入离心管以 1000r/min—1200r/min 的速度离心 5min—7min，弃去上清液，加入 0.075mol/L KC1 溶液低渗处理，继而以新配制的甲醇和冰醋酸液（容积比为 3:1）进行固定。空气干燥或火焰干燥法制片常规制片，用姬姆萨染液染色。

5.2.6 作染色体分析时，对化妆品终产品，每一处理组选择 100 个分散良好的中期分裂相（染色体数为 2n±2）进行染色体畸变分析。对化妆品原料，则每一处理组选 200 个（阳性对照可选 100 个）。在分析时应记录每一观察细胞的染色体数目，对于畸变细胞还应记录显微镜视野的坐标位置及畸变类型。

5.3 统计处理：对染色体畸变细胞率用 X2 检验，以评价受试物的致突变性。

5.4 结果评价：在下列两种情况下可判定受试物在本试验系统中具有致突变性：

（1）受试物引起染色体结构畸变数具有统计学意义，并有剂量相关性。

（2）受试物在任何一个剂量条件下，引起具有统计学意义的增加，并有可重复性。在评价时应把生物学和统计学意义结合考虑。

5.2.5 When harvesting the cells, digest the cells with 0.25% trypsin solution. After the cells fall off, add the culture medium containing 10% fetal bovine or calf serum to stop the trypsin effect, mix well, put it into a centrifuge tube and centrifugate at the speed of 1000r / min-1200r / min for 5min-7min, discard the supernatant and add 0.075mol/l KC1The solution is treated with low permeability, and then fixed with newly prepared methanol and glacial acetic acid solution (volume ratio of 3:1).The films are made by air drying or flame drying and dyed with Giemsa dye solution.

5.2.6 For chromosomal analysis, 100 well dispersed metaphase (chromosome number 2n ± 2) are selected for chromosomal aberration analysis in each treatment group. For cosmetic raw materials, 200 are selected for each treatment group (100 for positive control).The chromosome number of each observed cell should be recorded during the analysis, and the coordinate position and distortion type of the microscopical field of vision should also be recorded for the aberrant cells.

5.3 Statistical treatment: x2 test is used to evaluate the mutagenicity of the test substance.

5.4 Results evaluation: the mutagenicity of the test substance in the test system can be determined under the following two conditions:

(1) The number of chromosomal structural aberrations caused by the test substance is statistically significant and dose-dependent.

(2) Under any dose condition, the test substance causes a statistically significant increase and has repeatability. Biological and statistical significance should be considered in the evaluation.

6 结果解释

阳性结果表明受试物引起培养的哺乳动物体细胞染色体结构畸变。

阴性结果表明在本试验条件下，受试物不引起培养的哺乳动物体细胞染色体结构畸变。

6 Interpretation of results

The positive results showed that the test substance caused chromosome structural aberrations in cultured mammalian somatic cells.

The negative results showed that under the conditions of this experiment, the test substance did not cause the chromosome structure distortion of the cultured mammalian somatic cells.

10 体外哺乳动物细胞基因突变试验

In Vitro Mammalian Cell Gene Mutation Test

1 范围

本规范规定了体外哺乳类细胞基因突变试验的基本原则、要求和方法。本规范适用于检测化妆品原料及其产品的致突变性。

2 试验目的

该测试系统用于检测化妆品原料及其产品引起的突变，包括碱基对突变、移码突变和缺失等，从而评价受试物引起突变的可能性。

10 In Vitro Mammalian Cell Gene Mutation Test

1 Range

This specification specifies the basic principles, requirements and methods of gene mutation test of mammalian cells in vitro. This specification is applicable to the detection of mutagenicity of cosmetic raw materials and products.

2 Test purpose

The test system is used to detect the mutation caused by cosmetic raw materials and products, including base pair mutation, frameshift mutation and deletion, so as to evaluate the possibility of mutation caused by the test substance.

3 定义

3.1 正向突变 forward mutation
从原型至突变子型的基因突变，这种突变可引起酶和功能蛋白的改变。

3.2 突变频率 mutant frequency
所观察到的突变细胞数与存活细胞数之比值。

4 试验原理

在加入和不加入代谢活化系统的条件下，使细胞暴露于受试物一定时间，然后将细胞再传代培养。胸苷激酶正常水平的细胞对三氟胸苷（trifluorothymidine，TFT）等敏感，因而在培养液中不能生长分裂，突变细胞则不敏感，在含有 6-硫代鸟嘌呤（6-thioguanine，

6-TG）、 8-azaguanine (AG)或 TFT 的选择性培养液中能继续分裂并形成集落。基于突变集落数，计算突变频率以评价受试物的致突变性。

3 Definition

3.1 Forward mutation

Mutations in genes from prototypes to mutants that cause changes in enzymes and functional proteins.

3.2 Mutation frequency

The ratio of the number of mutant cells to the number of viable cells observed.

4 Test principle

Under the condition of adding or not adding the metabolic activation system, the cells are exposed to the test substance for a certain period of time, and then the cells are subcultured. The cells with normal thymidine kinase level are sensitive to trifluorotoxidine (TFT), so they can't grow and divide in the culture medium, while the mutant cells are not sensitive. They can continue to divide and form colonies in the selective culture medium containing 6-thioguanine (6-TG), 8-azaguanine (Ag) or TFT. Based on the number of mutation colonies, the mutation frequency is calculated to evaluate the mutagenicity of the test substance.

5 试验方法

5.1 试剂和受试物制备

5.1.1 受试物

5.1.1.1 受试物的配制：固体受试物需溶解或悬浮于溶剂中，用前稀释至适合浓度；液体受试物可以直接加入试验系统/或用前稀释至适合浓度。受试物应在使用前新鲜配制，否则就必须证实储存不影响其稳定性。

5.1.1.2 溶剂的选择：溶剂必须是非致突变物，不与受试物发生化学反应，不影响细胞存活和 S9 活性。首选溶剂是水或水溶性溶剂。二甲基亚砜（DMSO）也是常用溶剂，但使用时浓度不应大于 0.5%。

5.1.1.3 受试物浓度设置

5.1.1.3.1 最高浓度的选择：决定最高浓度的因素是细胞毒性、受试物在试验系统中的溶解度以及 pH 或渗克分子浓度（osmolality）的改变。

5.1.1.3.2 细胞毒性的确定：应使用指示细胞完整性和生长情况的指标，在活化系统存在或不存在两种条件下确定细胞毒性，例如相对集落形成率或相对细胞总生长情况（ total growth）。应在预试验中确定细胞毒性和溶解度。

5 test method

5.1 Reagent and test substance preparation

5.1.1 Test substance

5.1.1.1 Preparation of test substance: the solid test substance shall be dissolved or suspended in the solvent and diluted to the appropriate concentration before use; the liquid test substance can be directly added to the test system / or diluted to the appropriate

concentration before use. The test substance shall be freshly prepared before use, otherwise it must be confirmed that storage does not affect its stability.

5.1.1.2 Selection of solvent: the solvent must be non mutagenic, not react with the test substance, not affect cell survival and S9 activity. The preferred solvent is water or water-soluble. DMSO is also a common solvent, but the concentration should not be more than 0.5%.

5.1.1.3 Test substance concentration setting

5.1.1.3.1 Selection of the highest concentration: the factors determining the highest concentration are cytotoxicity, solubility of the test substance in the test system and the change of pH or osmolality.

5.1.1.3.2 Determination of cytotoxicity: indicators indicating cell integrity and growth should be used to determine cytotoxicity in the presence or absence of an activation system, such as relative colony-forming rate or total cell growth
growth）。Cytotoxicity and solubility should be determined in the pre-test.

5.1.1.3.3 剂量设置

至少应设置 4 个可供分析的浓度。当有细胞毒性时，其浓度范围应包括从最大毒性至几乎无毒性。通常浓度间隔系数在 2-√ 10 之间。

对于那些细胞毒性很低的化合物，最高浓度应是 5μL/mL, 5mg/mL 或 0.01mol/L。

如最高浓度是基于细胞毒性，那么该浓度组的细胞相对存活率（相对集落形成率）或相对细胞总生长情况应为 10%—20%（不低于 10%）。

对于相对不溶解的物质，其最高浓度应达到或超过在细胞培养状态下的溶解度限值。最好在试验处理开始和结束时均评价溶解度，因为由于 S9 等的存在，试验系统内在暴露过程中溶解度可能发生变化。不溶解性可用肉眼鉴别，但沉淀不应影响观察。

5.1.2 对照：在每一项试验中，在代谢活化系统存在和不存在的条件下均应设阳性对照和阴性（溶剂）对照。

5.1.1.3.3 Dose setting

At least 4 concentrations for analysis shall be set. When there is cytotoxicity, the concentration range should include from maximum toxicity to almost no toxicity. Usually the concentration interval coefficient is between 2 and √ 10.

For those compounds with low cytotoxicity, the maximum concentration should be 5 μ L / ml, 5mg / ml or 0.01mol/l.

If the highest concentration is based on cytotoxicity, the relative survival rate (relative colony formation rate) or relative total cell growth of the concentration group should be 10% - 20% (not less than 10%).

For relatively insoluble substances, the highest concentration should reach or exceed the solubility limit in cell culture. It is best to evaluate solubility at the beginning and end of the test treatment as solubility may change during exposure within the test system due to the presence of S9, etc. The insolubility can be identified by naked eyes, but the precipitation should not affect the observation.

5.1.2 Control: in each test, positive control and negative (solvent) control should be set under the condition that metabolic activation system exists or does not exist.

5.1.2.1 阳性对照：当使用代谢活化系统时，阳性对照物必须是要求代谢活化、并能引起突变的物质 。 在没有代谢活化系 统时 ， 阳性对照物可用甲磺酸乙酯（ethyl methanesulfonate-EMS，HPRT 试验）、甲磺酸甲酯（methyl methanesulphonate，MMS,TK 试验），乙基亚硝基脲（ethyl nitrosourea-ENU，HPRT 试验）等。在有代谢活化系统时，可以使用 3- 甲基胆蒽（ 3-methylcholanthrene， HPRT 试验； TK 试 验）、 环磷酰胺（cyclophosphamide,TK 试验）N-亚硝基胍（N-nitroso-dimethylamine，HPRT 试验）、7,12-二甲基苯蒽（HPRT 试验）等。也可使用其他适宜的阳性对照物。

5.1.2.2 阴性对照物:阴性对照(包括溶剂对照)除不含受试物外，其他处理应与受试物相同。此外，当不具有实验室历史资料证实所用溶剂无致突变作用和无其他有害作用时，还应 设空白对照。

5.1.2.1 Positive control: When using metabolic activation system, the positive control must be a substance that requires metabolic activation and can cause mutation. When there is no metabolic activation system, the positive contrast substance can be ethyl methanesulfonate-EMS (HPRT test), methyl methanesulfonate (MMS,TK test), ethyl nitrosourea-ENU (HPRT test), etc. When there is a metabolic activation system, 3-methylcholanthrone (HPRT test; TK test), cyclophosphamide (TK test), n-nitrosodimethylamine (HPRT test), 7,12- dimethylbenzanthracene (HPRT test), etc. Other suitable positive controls may also be used.

5.1.2.2 Negative control substance: the negative control substance (including solvent control substance) shall be treated the same as the test substance except for the test substance. In addition, when there is no laboratory historical data to prove that the solvent used has no mutagenic effect or other harmful effects, a blank control should be set up.

5.1.3 细胞：HPRT 位点突变分析常用中国仓鼠肺细胞株（V-79）和中国仓鼠卵巢细胞株（CHO）。TK 位点突变分析常用小鼠淋巴瘤细胞株（L5178Y）和人类淋巴母细胞株（TK6）。细胞在使用前应进行有无支原体污染的检查。

5.1.4 培养液：应根据实验所用系统和细胞类型来选择适宜的培养基。对于 V-79 或 CHO 细胞，常用 MEM（Eagle）培养基加入 10%胎牛血清和适量抗菌素。对于 L5178Y 或 TK6 细胞，常用 RPMI 1640 培养基加入 10%马血清和适量抗菌素。

5.1.5 活化系统：同体外哺乳类细胞染色体畸变试验。

5.1.6 选择剂：6-硫代鸟嘌呤（6-TG）：建议使用终浓度为 5mg/mL—10μg/mL，用碳酸氢钠溶液配制（0.5%）。三氟胸苷（TFT）：建议使用终浓度为 3mg /mL。

5.1.7 预处理培养基: THMG/ THG

为减少细胞的自发突变率,在试验前，先将细胞加在含 THMG 的培养液中培养 24h，杀灭自发的突变细胞，然后将细胞再接种于 THG（不含氨甲喋呤的 THMG 培养液）中培养 1—3d。

THMG 含除培养液成份外的各物质终未浓度如下：

胸苷 5´10-6mol/L

次黄嘌呤 5´10-5mol/L

氨甲喋呤 4´10-7mol/L

甘氨酸 1´10-4mol/L

5.1.3 Cell: HPRT site mutation analysis is commonly used for Chinese hamster lung cell strain (V-79) and chinese hamster ovary strain (CHO). TK site mutation analysis is commonly used for mouse lymphoma cell line (L5178Y) and human

lymphoblastic cell line (TK6). Cells should be checked for mycoplasma contamination before use.

5.1.4 Culture medium: the appropriate culture medium should be selected according to the system and cell type used in the experiment.For V-79 or CHO cells, 10% fetal bovine serum and appropriate amount of antibiotics are added to MEM (Eagle) medium. For L5178Y or TK6 cells, RPMI 1640 medium is used to add 10% horse serum and appropriate amount of antibiotics.

5.1.5 Activation system: in vitro mammalian cell chromosome aberration test.

5.1.6 Selector: 6-thioguanine (6-TG): it is recommended to use the final concentration of 5mg / ML-10 μ g / ml, prepared with sodium bicarbonate solution (0.5%).TFT: the recommended final concentration is 3mg / ml.

5.1.7 Pretreatment medium: thmg / THG

In order to reduce the spontaneous mutation rate of cells, before the test, the cells were added to THMG-containing culture solution for 24 hours to kill spontaneous mutant cells, and then the cells were inoculated into THG (THMG-containing culture solution without methotrexate) for 1-3 days.

The final concentration of THMG is as follows

Thymidine 5’10-6 mol/l

Hypoxanthine 5’10-5 mol/l

Methotrexate 4’10-7mol/l

Glycine 1’10-4mol/l

5.2 试验步骤

5.2.1 HPRT 位点突变分析

5.2.1.1 试验前 1d，接种细胞于培养瓶中，置于 37℃孵箱培养。

5.2.1.2 试验时吸去培养瓶中的培养液，加入一定浓度的受试物、S9-mix（不加入 S9-mix 的样品，用培养液补足）及一定量的不含血清培养液，置孵箱中处理 3h—6h 后，吸去培养液，用 Hank's 液洗细胞三次，加入含胎牛血清的培养液。

5.2.1.3 在受试物与细胞作用后当天和第 3d 将细胞按低密度分种，在第 7d 接种细胞，每个剂量 3 瓶。7d 后染色以测定细胞存活率。另将一定数量细胞接种于每个培养瓶中，每个剂量 8 瓶，3h 后加入 6-TG（终浓度为 5mg /mL），10d 后染色，计数突变细胞集落。

5.2 Test procedure

5.2.1 Mutation analysis of HPRT site

5.2.1.1 One day before the experiment, the cells are inoculated into culture flask and incubated in 37 ℃ incubator.

5.2.1.2 During the test, the culture solution in the culture flask was sucked out, and a certain concentration of the test substance, S9-mix (no S9-mix sample was added, and the culture solution was supplemented with the culture solution) and a certain amount of serum-free culture solution were added. after treatment for 3-6 hours in the incubator, the culture solution was sucked out, the cells were washed with Hank's solution three times, and the culture solution containing fetal bovine serum was added.

5.2.1.3 The cells are divided into low density groups on the same day and the third day after the interaction between the test substance and the cells. The cells are

inoculated on the seventh day with 3 bottles of each dose.7 days later, the cell survival rate is determined by staining. In addition, a certain number of cells are inoculated into each culture bottle, each dose is 8 bottles, and 6-TG is added 3 hours later (the final concentration is 5mg / ml), then stained 10 days later, and the mutant cell colonies are counted.

5.2.1.4 试验结果用 x 2 检验进行统计分析。

5.2.2 TK 位点突变分析（L5178Y 细胞，96 孔板法）

5.2.2.1 处理：取生长良好的细胞，调整密度为 5×105/mL，按 1%体积加入受试物，37℃震摇处理 3 小时。离心，弃上清液，用 PBS 或不含血清的培养基洗涤细胞 2 遍，重新悬浮细胞于含 10%马血清的 RPMI 1640 培养液中，并调整细胞密度为 2×105/mL。

5.2.2.2 PE0（0 天的平板接种效率）测定：取适量细胞悬液，作梯度稀释至 8 个细胞/mL，接种 96 孔板（每孔加 0.2 mL，即平均 1.6 个细胞/孔），每个剂量作 1—2 块板，37℃，5% CO2，饱和湿度条件下培养 12d，计数每块平板有集落生长的孔数。

5.2.2.3 表达：步骤 6.2.2.1 所得细胞悬液作 2d 表达培养，每天计数细胞密度并保持密度在 10_6/ml 以下。

5.2.1.4 The test results are analyzed by x2 test.

5.2.2 TK site mutation analysis (L5178Y cells, 96 well plate method)

5.2.2.1 Treatment: take the cells with good growth, adjust the density to 5 × 105 / ml, add the test substance by 1% volume, shake at 37 ℃ for 3 hours.After centrifugation, the supernatant is discarded and ished twice with PBS or serum-free medium, the cells are resuspended in RPMI 1640 medium containing 10% horse serum, and the cell density is adjusted to 2 × 105 / ml.

5.2.2.2 PE0(0-day plate inoculation efficiency) determination: take appropriate cell suspension, make gradient dilution to 8 cells /mL, inoculate 96-well plate (0.2 mL per well, i.e. 1.6 cells/well on average), make 1-2 plates for each dose, culture for 12 days at 37℃, 5% CO2 and saturated humidity, and count the number of wells with colony growth on each plate.

5.2.2.3 Expression: the cell suspension obtained in step 6.2.2.1 is cultured for 2D expression, and the cell density is counted every day and kept below 10_6/ ml.

5.2.2.4 PE2（第 2d 的平板接种效率）测定：第 2d 表达培养结束后，取适量细胞悬液，按步骤 6.2.2.2 作梯度稀释并接种 96 孔板，培养 12d 后计数每块平板有集落生长的孔数。

5.2.2.5 TFT 抗性突变频率（MF）测定：第 2d 表达培养结束后，取适量细胞悬液，调整细胞密度为 1×104/mL，加入 TFT（三氟胸苷，终浓度为 3μg/mL），混匀，接种 96 孔板（每孔加 0.2 mL，即平均 2000 个细胞/孔），每个剂量作 2—4 块板，37℃，5% CO2，饱和湿度条件下培养 12d，计数有突变集落生长的孔数。

5.2.2.4 Pe2 (the second day of plate inoculation efficiency) measurement: after the second day of expression and culture, take appropriate amount of cell suspension, make gradient dilution according to step 6.2.2.2 and inoculate 96 well

plates, and count the number of holes with colony growth in each plate after 12 days of culture.

5.2.2.5 Determination of TFT resistance mutation frequency (MF): after the end of 2d expression culture, take appropriate amount of cell suspension, adjust the cell density to 1×104/mL, add TFT (trifluorothymidine, final concentration is 3g/ml), mix well, inoculate 96-well plate (0.2 mL per well, i.e. an average of 2000 cells/well), make 2-4 plates for each dose, culture at 37℃, 5% CO2 under saturated humidity for 12 days, and count the number of wells with mutation colony growth.

5.2.2.6 计算

5.2.2.6.1 平板效率（PE0 和 PE2）

$$PE = \frac{-\ln（EW/TW）}{1.6}$$

式中：EW 为无集落生长的孔数；TW 为总孔数；

1.6 为每孔接种细胞数

5.2.2.6.2 相对存活率（RS%）

$$相对存活率（RS\%）= \frac{PE0（处理）}{PE0（对照）} \times 100\%$$

5.2.2.6.3 突变频率（MF）

$$MF(\times 10\text{-}6) = \frac{-\ln（EW/TW）/n}{PE2}$$

式中：EW 为无集落生长的孔数；TW 为总孔数；

n 为每孔接种细胞数（2000）

5.2.2.6 Calculation

5.2.2.6.1 Plate efficiency (Pe0 and Pe2)

$$PE = \frac{-\ln（EW/TW）}{1.6}$$

Where: EW is the number of pores without colony growth; TW is the total number of pores;

1.6 Number of cells inoculated per pore

5.2.2.6.2 Relative survival rate (RS%)

$$Relative\ survival\ rate\ (RS\%) = \frac{Pe0\ (processing)}{Pe0\ (control)} * 100\%$$

5.2.2.6.3 Mutation frequency (MF)

$$MF(\times 10\text{-}6) = \frac{-\ln（EW/TW）/n}{PE2}$$

Where: EW is the number of pores without colony growth; TW is the total number of pores;
N is the number of cells inoculated per pore (2000)

6 结果评价

在下列两种情况下可判定受试物在本试验系统中为阳性结果：
(1) 受试物引起突变频率具有统计学意义、并与剂量相关的增加。
(2) 受试物在任何一个剂量条件下，引起具有统计学意义，并有可重复性的阳性反应。阳性结果表明受试物可引起所用哺乳类细胞的基因突变。可重复的阳性剂量—反应关系意义更大。阴性结果表明在本试验条件下，受试物不引起所用哺乳类细胞的基因突变。

6 Result evaluation

In the following two cases, it can be determined that the test substance is a positive result in this test system:
(1) The frequency of mutation caused by test substance is statistically significant and dose-related.
(2) Under any dose condition, the tested substance causes a positive reaction with statistical significance and repeatability. Positive results show that the tested substance can cause gene mutation of mammalian cells used. Repeated positive dose-response relationship is of greater significance. Negative results showed that under the test conditions, the tested substance did not cause gene mutation in the mammalian cells used.

11 哺乳动物骨髓细胞染色体畸变试验

In Vivo Mammalian Bone Marrow Cell Chromosome Aberration Test

1 范围

本规范规定了哺乳动物骨髓细胞染色体畸变试验的基本原则、要求和方法。本规范适用于检测化妆品原料及其产品的遗传毒性。

2 试验目的

本试验是一项致突变性试验，检测整体动物骨髓细胞染色体畸变，以评价受试物致突变的可能性。

11 In Vivo Mammalian Bone Marrow Cell Chromosome Aberration Test

1 Range

This specification specifies the basic principles, requirements and methods of chromosome aberration test for mammalian bone marrow cells. This specification is applicable to the detection of genetic toxicity of cosmetic raw materials and products.

2 Test purpose

This test is a mutagenicity test to detect chromosomal aberrations of bone marrow cells of whole animals, so as to evaluate the possibility of mutagenicity of test substance.

3 定义

3.1 染色体型畸变 chromosome-type aberration

染色体结构损伤，表现为在两个染色单体的相同位点均出现断裂或断裂重组的改变。

3.2 染色单体型畸变 chromatid-type aberration

染色体结构损伤，表现为染色单体断裂或染色单体断裂重组的损伤。

3.3 染色体数目畸变 numerical-type aberration

哺乳动物细胞染色体数目的改变。

3 Definition

3.1 chromosome-type aberration

The damage of chromosome structure is manifested by the change of breakage or recombination at the same site of two chromatids.

3.2 chromatid-type aberration

The damage of chromosome structure is the damage of chromatid breaking or chromatid breaking and recombination.

3.3 numerical-type aberration

Changes of chromosome number in mammalian cells.

4 试验基本原则

使哺乳动物（如大鼠或小鼠）经口或其他适宜途径染毒，动物处死前用细胞分裂中期阻断剂处理，处死后制备骨髓细胞染色体标本，分析染色体畸变。

本方法特别适用于需考虑体内代谢活化后的染色体畸变分析。

若有证据表明待测物或其代谢产物不能到达骨髓，则不适用于本方法。

4 Basic principles of test

To infect mammals (such as rats or mice) orally or by other appropriate ways. Before the animals are killed, they are treated with the medium-term blocker of cell division. After the animals are killed, the chromosome samples of bone marrow cells are prepared and the chromosome aberrations are analyzed.

This method is especially suitable for the analysis of chromosomal aberrations after metabolic activation in vivo.

If there is evidence that the substance to be tested or its metabolites cannot reach the bone marrow, this method is not applicable.

5 试验方法

5.1 实验动物和饲养环境：

选用健康成年啮齿类动物，推荐使用大鼠或小鼠，每组每种性别至少 5 只，动物在实验室中至少应适应 5 天，实验开始时每一性别动物的体重差异应控制在±20%内。实验动物及实验动物房应符合国家相应规定。

5 test method

5.1 Laboratory animals and feeding environment:

Healthy adult rodents are selected, and rats or mice are recommended. There are at least 5 animals of each sex in each group. The animals should be adapted for at least 5 days in the laboratory. At the beginning of the experiment, the weight difference of animals of each sex should be controlled within+/-20%. Laboratory animals and laboratory animal houses shall conform to the corresponding regulations of the state.

5.2 受试物

5.2.1 受试物配制：固体受试物应溶解或悬浮于适合的溶剂中，并稀释至一定浓度。液体受试物可直接使用或予以稀释。受试物应在使用前新鲜配制，否则就必须证实贮存不影响其稳定性。

5.2.2 溶剂的选择：溶剂在所选用浓度下，不引起毒性效应，不与受试物发生化学反应。水为首选溶剂。

5.2.3 剂量设置：应进行预试验以选择最高剂量。当有毒性时，可以引起死亡或者抑制骨髓细胞有丝分裂指数（50%以上）为指标确定最高剂量。在第一次采集样品时，需设置 3 个可供分析的剂量，在第二次采集样品时，则仅需设置最高剂量组。

如果一次剂量为 2000mg/kg 体重时仍未引起毒性效应，则只设 2000mg/kg 体重剂量组。如果人类的可能（期望）暴露量过大，可选择 2000mg/kg/BW/d 染毒 14 天，或选择 1000mg/kg/B W/d 染毒大于 14 天进行试验。

5.2 Test substance

5.2.1 Preparation of test substance: the solid test substance shall be dissolved or suspended in a suitable solvent and diluted to a certain concentration. The liquid test substance can be used directly or diluted. The test substance shall be freshly prepared before use, otherwise it must be confirmed that storage does not affect its stability.

5.2.2 Selection of solvent: under the selected concentration, the solvent will not cause toxic effect or chemical reaction with the test substance. Water is the preferred solvent.

5.2.3 Dose setting: a pretest should be performed to select the highest dose. When it is toxic, it can cause death or inhibit the mitosis index of bone marrow cells (more than 50%) to determine the maximum dose. In the first sample collection, three doses for analysis need to be set. In the second sample collection, only the highest dose group needs to be set.

If a single dose of 2000mg/kg body weight does not cause toxic effects, only a dose group of 2000mg/kg body weight is set. If the possible (expected) exposure of human is too large, the test can be carried out by selecting 2000mg/kg/BW/d for 14 days or 1000mg/kg/B W/d for more than 14 days.

5.3 对照：在每项试验中，对每种性别均应设阴性对照组和阳性对照组。除不使用受试物外，其他处理与受试物组一致。

5.3.1 阴性对照：除设溶剂对照（即仅含溶剂）外，如果没有文献资料或历史性资料证实所用溶剂不具有有害作用或致突变作用，还应设空白对照组。

5.3.2 阳性对照：阳性对照物应能引起染色体结构畸变率明显高于背景资料。染毒途径可以不同于受试物。所选用的阳性对照物最好与受试物类别有关。可以使用下述物质：三亚乙基密胺（triethylenemelamine）、甲磺酸乙酯（ethyl methanesulphonate）、乙基亚硝基脲（ethylnitrosonrea）、丝裂霉素 C（mytomycin C）和环磷酰胺（cyclophosphamide）。

5.3 Control: in each test, negative control group and positive control group shall be set for each sex.The other treatments are the same as the test substance group except that the test substance is not used.

5.3.1 Negative control: in addition to solvent control (i.e. only containing solvent), if there is no literature or historical data to prove that the solvent used does not have harmful or mutagenic effects, a blank control group should also be set.

5.3.2 Positive control: Positive control should cause chromosome structural aberration rate significantly higher than background data. The route of exposure may be different from that of the subject. The selected positive control substance is preferably related to the type of test substance. The following substances can be used: triethylenemelamine, ethyl methanesulphonate, ethylnitrosonrea, Mitomycin C and cyclophosphamide.

5.4 染毒方式：可采用经口或其他适宜的染毒方式。一般染毒为一次完成，如剂量过大时，一天内染毒数次也是可以的，但每次应间隔数小时。

一般情况下，染毒 1 次，但分两次采集标本,即每组动物分两个亚组，亚组 1 于染毒后 12h—18h 处死并采集第一次标本；亚组 2 于亚组 1 处死后 24h 采集第二次标本。如果采用多次染毒，于末次染毒后 12h—18h 采集标本。于处死动物采集标本前腹腔注射细胞分裂中期阻断剂（如用秋水仙素，于处死前 4h，按 4mg/kg 体重给药。若使用动物为小鼠，适宜的处理时间为 3—5 h，若使用动物为中国仓鼠，适宜的处理时间为 4—5 h。

5.4 Method of poisoning: oral or other appropriate methods can be used. Generally, the poisoning is completed at one time. If the dosage is too large, it is OK to be poisoned several times a day, but the interval should be several hours each time.

In general, the animals in each group are divided into two subgroups. Subgroup 1 is collected after the poisoning

In general, the animals were exposed once, but the specimens were collected twice, i.e. each group of animals was divided into two subgroups. subgroup 1 was killed 12-18 hours after exposure and the first specimen was collected. Subgroup 2 collected the second sample 24 hours after subgroup 1 was executed. If multiple exposures are used, specimens are collected 12-18 hours after the last exposure. Cell division metaphase blockers (such as colchicine, 4mg/kg body weight) were injected intraperitoneally before the animals were killed to collect specimens. If the animal used is a mouse, the appropriate treatment time is 3-5 h, and if the animal used is a Chinese hamster, the appropriate treatment time is 4-5 h.

5.5 试验步骤

5.5.1 用颈椎脱臼法处死动物，取出股骨，剔除肌肉等组织。

5.5.2 剪去股骨两端，用注射器吸取 5mL 生理盐水，从股骨一端注入，用 10mL 离心管，从股骨另一端接取流出的骨髓细胞悬液。

5.5.3 将细胞悬液以 1000r/min 的速度离心 5 min—7 min，去除上清液。

5.5.4 加入 0.075mol/L KCl 溶液 7ml，用滴管将细胞轻轻地混匀，放入 37℃水浴中低渗处理 7min，加入 1—2mL 固定液（冰醋酸:甲醇=1:3），混匀，以 1000r/min 速度离心 5 min—7min，弃去上清液。

5.5.5 加入 7mL 固定液，混匀，固定 15min，以 1000r/min 的速度离心 7min，弃去上清液。

5.5.6 用同法再固定 1—2 次，弃去上清液。

5.5.7 加入数滴新鲜固定液，混匀。

5.5.8 用混悬液以空气干燥或火焰干燥法制片。

5.5.9 用姬姆萨染液染色。

5.5 Test procedure

5.5.1 Animals are killed by cervical dislocation, femurs are removed and muscles are removed.

5.5.2 Cut off both ends of the femur, use a syringe to suck 5ml of normal saline, inject it from one end of the femur, use a 10ml centrifuge tube, and connect the marrow cell suspension from the other end of the femur.

5.5.3 The cell suspension is centrifuged at a rate of 1000 R / min for 5-7 min to remove the supernatant.

5.5.4 Add 7ml of 0.075mol/l KCl solution, mix the cells gently with a burette, put them into a 37 ℃ water bath for 7 min with low permeability treatment, add 1-2ml of fixed solution (glacial acetic acid: methanol = 1:3), mix them evenly, centrifugate at a speed of 1000r / min for 5-7min, and discard the supernatant.

5.5.5 Add 7ml of fixing liquid, mix well, fix for 15min, centrifugate at the speed of 1000r / min for 7min, and discard the supernatant.

5.5.6 Use the same method to fix again 1-2 times, and discard the supernatant.

5.5.7 Add a few drops of fresh fixative and mix well.

5.5.8 The suspension is used for air drying or flame drying.

5.5.9 Dye with Giemsa dye.

5.6.1 确定有丝分裂指数：包括所有处理组、阳性和阴性对照组（每只动物计数 500—1000 个细胞）。

5.6.2 计数畸变细胞：对每只动物至少选择 100 个分散良好的中期分裂相，在显微镜油镜下进行读片。由于低渗等机械作用的破坏，会导致处于中期的染色体发生丢失，所以，观察的中期相染色体数目应控制在 2n±2 内。在读片时应记录每一观察细胞的染色体数目，对于畸变细胞还应记录显微镜视野的坐标位置及畸变类型。裂隙（Gap）应单独记录并列出，通常不作为染色体结构畸变计算。所得各组的染色体畸变率用 X2 检验等进行统计学处理，以评价试验组和对照组之间是否有显著差异。

5.6.1 Determine mitotic index: including all treatment groups, positive and negative control groups (500-1000 cells per animal).

5.6.2 Count aberrant cells: select at least 100 well dispersed metaphase of each animal, and read the film under the microscope oil microscope. The number of metaphase chromosomes should be controlled within 2n ± 2 because of the loss of metaphase chromosomes due to the mechanical effects such as hypotonic. The chromosome number of each observation cell should be recorded during the reading of the film, and the coordinate position and distortion type of the microscopical field of vision should also be recorded for the aberrant cells. Gap should be recorded and listed separately, which is not usually used as the calculation of chromosome structural aberrations. In order to evaluate whether there is significant difference between the experimental group and the control group.

5.7 结果评价

每个动物作为一个试验单位，在统计分析时，每个动物的数据应列表进行。可把结构畸变细胞率（%）和每细胞内的染色体畸变数作为评价指标。统计分析的标准有几个，当受试物引起染色体畸变数具有统计学意义，并有与剂量相关的增加或者在一个剂量组、单一时间点采样的试验中出现染色体畸变细胞数明显增高，则判定具有致突变性。

在评价时应综合考虑生物学意义和统计学意义，不能作出明确结论时，应改变试验条件进一步进行测试。

5.7 Result evaluation

Each animal as a test unit, in statistical analysis, the data of each animal should be tabulated. The cell rate (%) of structural aberration and chromosome aberration per cell can be used as evaluation indexes. There are several criteria for statistical analysis. Mutagenicity is determined when the number of chromosome aberration cells caused by the test object has statistical significance, and there is a dose-related increase or the number of chromosome aberration cells is significantly increased in a dose group and a single time point sampling test.

The biological and statistical significance should be taken into account in the evaluation, and the test conditions should be changed for further testing if no clear conclusion can be made.

6 结果解释

阳性结果证明受试物具有引起该种受试动物骨髓细胞染色体畸变的能力。

阴性结果表明在本试验条件下受试物不引起该种受试动物骨髓细胞染色体畸变。

6 Interpretation of results

The positive results showed that the test substance had the ability to cause chromosomal aberrations in bone marrow cells of this kind of animal.

The negative results showed that the test substance did not cause chromosomal aberrations of bone marrow cells of this kind of animal under the test conditions.

12 体内哺乳动物细胞微核试验

Mammalian Erythrocyte Micronucleus Test

1 范围

本规范规定了哺乳动物红细胞微核试验的基本原则、要求和方法。本规范适用于化妆品原料的染色体畸变检测。

2 定义

微核 micronucleus

染色单体或染色体的无着丝点断片，或因纺锤体受损而丢失的整个染色体，在细胞分裂后期，仍然遗留在细胞质中。末期之后，单独形成一个或几个规则的次核，被包含在子细胞的胞质内，因比主核小，故称为微核。

12 Mammalian Erythrocyte Micronucleus Test

1 Range

This specification specifies the basic principles, requirements and methods of mammalian micronucleus test. This specification is applicable to chromosome aberration detection of cosmetic raw materials.

2 Definition

Micronucleus

At the later stage of cell division, the chromatid or chromosome acentric fragment, or the whole chromosome lost due to the damage of spindle, is still left in the cytoplasm. After the end of the period, one or several regular subnuclei are formed, which are

contained in the cytoplasm of daughter cells. Because they are smaller than the main nucleus, they are called micronuclei.

3 原理

凡能使染色体发生断裂或使染色体和纺锤体联结损伤的化学物，都可用微核试验来检测。各种类型的骨髓细胞都可形成微核，但有核细胞的胞质少，微核与正常核叶及核的突起难以鉴别。嗜多染红细胞是分裂后期的红细胞由幼年发展为成熟红细胞的一个阶段，此时红细胞的主核已排出，因胞质内含有核糖体，姬姆萨染色呈灰蓝色，成熟红细胞的核糖体已消失，被染成淡桔红色。骨髓中嗜多染红细胞数量充足，微核容易辨认，而且微核自发率低，因此，骨髓中嗜多染红细胞成为微核试验的首选细胞群。

若动物染毒的时间达 4 周以上，也可选同一终点的外周血正染红细胞进行微核试验。若有证据表明待测物或其代谢产物不能到达骨髓，则不适用于本方法。

3 principle

Micronucleus test can be used to detect chemicals that can break chromosomes or damage the connection between chromosomes and spindles. All kinds of bone marrow cells can form micronucleus, but the cytoplasm of nucleated cells is few, and it is difficult to distinguish micronucleus from normal nuclear leaves and nuclear processes. Polychromatic erythrocytes are a stage in which erythrocytes develop from infancy to mature erythrocytes at the later stage of division. At this time, the main nucleus of erythrocytes has been discharged. Because ribosomes are contained in the cytoplasm, Giemsa staining is gray blue, and the ribosomes of mature erythrocytes have disappeared and dyed light orange red. The number of polychromatic erythrocytes in bone marrow is sufficient, the micronucleus is easy to identify, and the spontaneous rate of micronucleus is low. Therefore, polychromatic erythrocytes in bone marrow become the preferred cell group for micronucleus test.

If the exposure time of the animal is more than 4 weeks, the micronucleus test can also be performed on the peripheral blood positive erythrocytes at the same end point. If there is evidence that the substance to be tested or its metabolites cannot reach the bone marrow, this method is not applicable.

4 试验的基本原则

通过适当的途径使动物接触受试物，一定时间后处死动物，取出骨髓，制备涂片，经固定、染色，在显微镜下计数含微核的嗜多染红细胞。

5 仪器和器械

生物显微镜、解剖剪、镊子、止血钳、注射器、灌胃针头、载玻片、盖玻片（24mm×50mm）、塑料吸瓶、纱布、滤纸等。

4 Basic principles of test

The animals are exposed to the test substance through appropriate ways. After a certain period of time, the animals are killed, the bone marrow is taken out, the smear is prepared, fixed and stained, and the polychromatic erythrocytes containing micronucleus are counted under the microscope.

5 Instruments and apparatus

Biological microscope, dissecting scissors, forceps, hemostatic forceps, syringes, stomach filling needles, slides, cover slides (24mm × 50mm), plastic suction bottles, gauze, filter paper, etc.

6 试剂

6.1 小牛血清（灭活）

将滤菌的小牛血清置于 56℃恒温水浴保温 30min 灭活。灭活的小牛血清通常保存于冰箱冷冻室里。

6.2 姬姆萨（Giemsa）染液

成分：Giemsa 染料 3.8g

甲醇 375mL

甘油 125mL

配制：将染料和少量甲醇于乳钵里仔细研磨，再加入甲醇至 375mL 和甘油，混合均匀，放置 37℃恒温箱中保温 48h。保温期间，振摇数次，促使染料的充分溶解，取出过滤，两周后用。

6.3 1/15mol/L 磷酸盐缓冲液（pH6.8）

磷酸二氢钾（KH2PO4） 4.50 g

磷酸氢二钠（Na2HPO4·12H2O） 11.81 g

加蒸馏水至 1000mL

6.4 Giemsa 应用液

取一份 Giemsa 染液与 6 份 1/15mol/L 磷酸盐缓冲液混合而成。现用现配。

6 reagent

6.1 Calf serum (inactivated)

The calf serum is inactivated in a constant temperature water bath at 56 ℃ for 30 minutes. Inactivated calf serum is usually stored in a refrigerator freezer.

6.2 Giemsa dye solution

Ingredient: Giemsa dye 3.8g

Methanol 375ml

Glycerin 125ml

Preparation: carefully grind the dye and a small amount of methanol in a mortar, add methanol to 375ml and glycerin, mix evenly, and place in a 37 ℃ incubator for 48h.During the heat preservation period, shake several times to make the dye fully dissolved, take it out and filter it, and use it two weeks later.

6.3 1 / 15mol / L phosphate buffer (ph6.8)

Potassium dihydrogen phosphate (KH2PO4) 4.50 G

Disodium hydrogen phosphate (Na2HPO4 · 12H2O) 11.81 G

Add distilled water to 1000ml

6.4 Giemsa solution

One part of Giemsa dye solution is mixed with six parts of 1 / 15mol / L phosphate buffer solution. Matching only before use.

7 实验动物和饲养环境

适宜的哺乳动物均适用于本实验，推荐使用小鼠或大鼠。小鼠是微核试验的常规动物。体重为 25g —30g。也可选用成年大鼠，体重为 150g —200g。动物在实验室中至少应适应 3-5 天，实验开始时每一性别动物的体重差异应控制在±20%内。

实验动物及实验动物房应符合国家相应规定。

7 Laboratory animals and feeding environment

Suitable mammals are suitable for this experiment, and mice or rats are recommended. Mice are routine animals for micronucleus test. The body weight is 25g-30g.Adult rats with a weight of 150g-200g can also be selected. The animals should adapt for at least 3-5 days in the laboratory. At the beginning of the experiment, the weight difference of each sex should be controlled within ± 20%.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations.

8 剂量分组

一般取受试物 LD50 的 1/2、1/5、1/10、1/20 等剂量，以求获得微核的剂量-反应关系曲线。当受试物的 LD50 大于 5g/kg 体重时，可取 5g/kg 体重为最高剂量，一般至少设 3 个剂量。每个剂量组 10 只动物，雌、雄性各半。另外，还应设溶剂对照和阳性物对照组。常用环磷酰胺作为阳性物对照，剂量可为 40mg/kg 体重。

如果人类的可能（期望）暴露量过大，可选择 2000mg/kg/BW/d 染毒 14 天，或选择 1000mg/kg/B W/d 染毒大于 14 天进行试验。

根据受试物的理化性质（水溶性和/或脂溶性）确定受试物所用的溶剂，通常用水、植物油或食用淀粉等。

8 Dose grouping

Generally, 1 / 2, 1 / 5, 1 / 10 and 1 / 20 of LD50 are taken to obtain the dose-response curve of micronucleus. When LD50 of the test substance is greater than 5g / kg body weight, 5g / kg body weight can be taken as the highest dose, generally at least 3 doses are set.10 animals in each dose group, half female and half male. In addition, a solvent control group and a positive control group should be set up. Cyclophosphamide is commonly used as a positive control, and the dosage can be 40mg / kg body weight.

If the possible (expected) exposure of human is too large, the test can be carried out by selecting 2000mg/kg/BW/d for 14 days or 1000mg/kg/B W/d for more than 14 days.

According to the physical and chemical properties (water-soluble and / or fat soluble) of the test object, the solvent used for the test object is determined, usually water, vegetable oil or edible starch, etc.

9 染毒途径和方式

染毒途径视实验目的而定，建议采用经口灌胃方式。采用 30h 两次给药法，即两次给受试物间隔 24h，第二次给受试物后 6h 取材。

10 试验方法

10.1 样本的制取

动物颈椎脱臼处死后，打开胸腔，沿着胸骨柄与肋骨交界处剪断，剥掉附着其上的肌肉，擦净血污，横向剪开胸骨，暴露骨髓腔，然后用止血钳挤出骨髓液。

长时间染毒的外周血样本从尾或耳静脉采血，一般应在未次染毒的 18—24h、36—48h 之间分两次进行。

10.2 涂片

将骨髓液滴在载玻片一端的小牛血清液滴里，仔细混匀。一般来讲，两节胸骨髓液涂一张片子为宜。然后，按血常规涂片法涂片，长度约 2cm —3cm。在空气中晾干。若立即染色，需在酒精灯火焰上方，稍微烘烤一下。

9 Ways and means of poisoning

The route of poisoning depends on the purpose of the experiment, and oral gavage is recommended. The drug is given twice in 30h, i.e. the interval between two times is 24h, and the material is taken 6h after the second time.

10 test method

10.1 Sample preparation

After cervical vertebrae dislocated and killed, open the chest cavity, cut along the junction of the sternal stalk and rib, strip the muscle attached to it, wipe off the blood stain, cut the sternum horizontally, expose the marrow cavity, and then use hemostatic forceps to extrude the marrow fluid.

Peripheral blood samples exposed for a long time should be collected from tail or ear vein in two times between 18-24 hours and 36-48 hours without secondary exposure.

10.2 smear

Drop the marrow into the calf serum drop at one end of the slide and mix it carefully. Generally speaking, it's better to apply one film of two sections of thoracic marrow fluid. Then, smear according to the blood routine smear method, the length is about 2cm-3cm.Dry in the air. If dyeing immediately, it is necessary to bake slightly above the flame of alcohol lamp.

10.3 固定

将干燥的涂片放入甲醇液中固定 5min。即使当日不染色，也应固定后保存。

10.4 染色

将固定过的涂片放入 Giemsa 应用液中，染色 10min —15min，然后立即用 1/15mol/L 磷酸盐缓冲液冲洗。

10.5 封片

用滤纸及时擦干染片背面的水滴，再用双层滤纸轻轻按压染片，以吸附染片上残留的水分，再在空气中晃动数次，以促其尽快晾干，然后放入二甲苯中透明 5min，取出滴上适量光学树脂胶，盖上盖玻片，写好标签。

10.6 观察与计数

先用低倍镜，后用高倍镜粗略检查，选择细胞分布均匀，细胞无损，着色适当的区域，再在油浸镜下计数。虽然不计数含微核的有核细胞，但需用有核细胞形态染色完好做好判断制片优劣的标准。

本法观察含微核的嗜多染红细胞。嗜多染红细胞呈灰蓝色，成熟红细胞呈淡桔红色。微核大多数呈单个圆形，边缘光滑整齐，嗜色性与核质相一致，呈紫红色或蓝紫色。

每只动物至少计数 2000 个嗜多染红细胞。微核率指含有微核的嗜多染红细胞数，以千分率（‰）表示之。若一个嗜多染红细胞中出现两个或两个以上微核，仍按一个有微核细胞计数。

经过化妆品标准委员会验证或证实的图像自动分析系统与流式细胞仪进行的微核试验，可接受为本方法的替代试验。

10.3 fixed

Put the dried smear into methanol solution and fix it for 5min. Even if it is not stained on that day, it should be fixed and stored.

10.4 dyeing

Put the fixed smear into Giemsa application solution, dye for 10min-15min, and then immediately wash it with 1 / 15mol / L phosphate buffer.

10.5 Seals

Wipe the water drop on the back of the dye pad with filter paper in time, press the dye pad gently with double-layer filter paper to absorb the residual water on the dye pad, shake it in the air for several times to promote it to dry as soon as possible, then put it into xylene for 5min, take out the appropriate amount of optical resin glue, cover the cover glass and write the label.

10.6 Observation and counting

First use low power microscope, then use high power microscope to roughly check, select the area with uniform cell distribution, no damage and proper coloring, and then count under oil immersion microscope. Although there is no count of nucleated cells with micronucleus, it is necessary to make a good standard to judge the quality of the production.

Polychromatic erythrocytes with micronucleus are observed. Polychromatic erythrocytes are gray blue, mature erythrocytes are light orange red. Most of the micronuclei are single and round, with smooth and neat edges. The color preference is consistent with the nucleoplasm, and they are purplish red or blue purple.

At least 2000 polychromatic erythrocytes are counted in each animal. Micronucleus rate refers to the number of polychromatic erythrocytes containing

micronucleus, expressed in thousands (‰).If there are two or more micronuclei in a polychromatic red blood cell, it is still counted as one with micronuclei.

The micronucleus test conducted by the image automatic analysis system and flow cytometer verified or confirmed by the cosmetics standards committee can be accepted as an alternative test of this method.

11 数据处理和结果判断

11.1 数据处理

报告各组微核细胞率的均数和标准差，利用适当的统计学方法如 Poinsson 分布 u 检验比较受试物各剂量组与溶剂对照组的微核率。

若无证据表明所得的数据有性别间的差异，则可将两性别的数据合并进行统计分析。

11.2 结果判定

在评价时应综合考虑生物学意义和统计学意义。如果受试物试验组与溶剂对照组相比，单一剂量法微核率有明显增高；多剂量法的剂量组在统计学上有显著性差异，并有剂量—反应关系则可认为微核试验阳性。

11 Data processing and result judgment

11.1 data processing

The mean and standard deviation of micronucleus cell rate of each group are reported. The micronucleus rate of each dose group is compared with that of the solvent control group by using appropriate statistical methods such as Poisson distribution U test.

If there is no evidence that the data obtained are different in sex, the data of gender can be combined for statistical analysis.

11.2 Result determination

Biological significance and statistical significance should be considered in the evaluation. If the micronucleus rate of the test substance test group is significantly higher than that of the solvent control group, the micronucleus rate of the multi dose test group is statistically significant, and there is a dose-response relationship, it can be considered that the micronucleus test is positive.

13 睾丸 Th 殖细胞染色体畸变试验

Testicle Cells Chromosome Aberration Test

1 范围

本规范规定了哺乳动物睾丸生殖细胞染色体畸变试验的基本原则、要求和方法。本规范适用于化妆品原料的遗传毒性检测。

2 试验目的

检测雄性动物生殖细胞染色体损伤，以评价受试物在生殖细胞诱导可遗传的致突变的可能性。

13 Testicle Cells Chromosome Aberration Test

1 Range

This specification specifies the basic principles, requirements and methods of chromosome aberration test for mammalian testicular germ cells. This specification is applicable to the genotoxicity test of cosmetic raw materials.

2 Test purpose

In order to evaluate the possibility of inducing heritable mutagenesis in germ cells, chromosome damage of germ cells of male animals is detected.

3 定义

3.1 染色体型畸变 chromosome-type aberration
染色体结构损伤，表现为两个染色单体的相同位点均出现断裂或断裂重接。

3.2 染色单体型畸变 chromatid-type aberration
染色体结构损伤，表现为染色单体断裂或染色单体断裂重接。

3.3 染色体数目畸变 numerical-type aberration
染色体数目发生改变，不同于正常二倍体核型，包括整倍体和非整倍体。

3 Definition

3.1 chromosome-type aberration
The damage of chromosome structure shows that the same sites of two chromatids are broken or reconnected.

3.2 chromatid-type aberration
The chromosome structure is damaged, which is manifested as chromatid breaking or chromatid breaking and reconnecting.

3.3 numerical-type aberration
The number of chromosomes changed, which is different from normal diploid karyotype, including aneuploid and aneuploid.

4 试验的基本原则

通过适当的途径使动物接触受试物，一定时间后处死动物，动物处死前用细胞分裂中期阻断剂处理，处死后制备睾丸初级精母细胞染色体标本，在显微镜下观察染色体畸变。

本方法特别适用于需考虑体内代谢活化后的染色体畸变分析。

若有证据表明待测物或其代谢产物不能到达睾丸，则不适用于本方法。

4 Basic principles of test

The animals are exposed to the test substance through appropriate ways. The animals are killed after a certain period of time. Before the animals are killed, they are treated with the medium-term cell division blocker. After the animals are killed, the chromosome samples of primary spermatocytes of testis are prepared, and the chromosome aberrations are observed under the microscope.

This method is especially suitable for the analysis of chromosomal aberrations after metabolic activation in vivo.

If there is evidence that the substance to be tested or its metabolites cannot reach the testis, this method is not applicable.

5 仪器和器械

生物显微镜、离心机、解剖剪、镊子、离心管、平皿、注射器、灌胃针头、载玻片、盖玻片（24mm×50mm）等。

5 Instruments and apparatus

Biological microscope, centrifuge, dissecting scissors, tweezers, centrifuge tubes, plates, syringes, stomach filling needles, slides, cover slides (24mm × 50mm), etc.

6 试剂

6.1 0.04%秋水仙素：取 40mg 秋水仙素，加生理盐水至 100mL。

6.2 1%柠檬酸三钠：取 1g 柠檬酸三钠，加蒸馏水至 100mL。

6.3 0.075mol/L 氯化钾溶液：取氯化钾 5.59g，加蒸馏水至 1000mL。

6.4 甲醇/冰醋酸（3：1，v/v）固定液：临用现配。

6.5 60%冰乙酸：取 60mL 冰乙酸，加蒸馏水至 100mL，均宜新鲜配制。

6.6 pH6.8 磷酸盐缓冲液

1/15mol/L 磷酸盐缓冲液（pH6.8）

磷酸二氢钾（KH_2PO_4） 4.50 g

磷酸氢二钠（$Na_2HPO_4 \cdot 12H_2O$） 11.81 g

加蒸馏水至 1000mL

6.7 姬姆萨染液

姬姆萨（Giemsa）染液

成分：Giemsa 染料 3.8g

甲醇 375mL

甘油 125mL

配制：将染料和少量甲醇于乳钵里仔细研磨，再加入甲醇至 375mL 和甘油，混合均匀，放置 37℃恒温箱中保温 48h。保温期间，振摇数次，促使染料的充分溶解，取出过滤，两周后用。

姬姆萨应用液：取 1mL 储备液加入 10mL pH6.8 磷酸缓冲液。

6.8 生理盐水、甲醇。

6 reagent

6.1 0.04% colchicine: take 40 mg colchicine and add normal saline to 100 ml.

6.2 1% trisodium citrate: take 1g trisodium citrate, add distilled water to 100ml.

6.3 0.075mol/l potassium chloride solution: take 5.59g of potassium chloride, add distilled water to 1000ml.

6.4 Fixed solution of methanol / glacial acetic acid (3:1, V / V): ready to use.

6.5 60% glacial acetic acid: take 60ml glacial acetic acid, add distilled water to 100ml, which should be prepared fresh.

6.6 Ph6.8 phosphate buffer

1 / 15mol / L phosphate buffer (ph6.8)

Potassium dihydrogen phosphate (KH2PO4) 4.50 G

Disodium hydrogen phosphate (Na2HPO4 · 12H2O) 11.81 G

Add distilled water to 1000ml

6.7 Giemsa dye

Giemsa dye

composition:

Giemsa dye 3.8g

Methanol 375ml

Glycerin 125ml

Preparation: carefully grind the dye and a small amount of methanol in a mortar, add methanol to 375ml and glycerin, mix evenly, and place in a 37 ℃ incubator for 48h.During the heat preservation period, shake several times to make the dye fully dissolved, take it out and filter it, and use it two weeks later.

Giemsa application solution: take LML stock solution and add 10ml ph6.8 phosphoric acid buffer solution.

6.8 Normal saline, methanol.

7 实验动物和饲养环境

适宜的雄性啮齿类动物均适用于本实验。推荐使用小鼠，6 周—8 周龄，体重为 30g—35g。动物在实验室中至少应适应 5 天，实验开始时动物的体重差异应控制在±20%内。实验动物及实验动物房应符合国家相应规定。

7 Laboratory animals and feeding environment

Suitable male rodents are all suitable for this experiment. It is recommended to use mice, 6-8 weeks old, weighing 30g—35g. Animals should adapt for at least 5 days in the laboratory, and the weight difference of animals should be controlled within+/-20% at the beginning of the experiment. Laboratory animals and laboratory animal houses shall conform to the corresponding regulations of the state.

8 剂量分组

受试物至少设三个剂量组。分别取 1/2、1/5、1/10 或 1/20LD50 剂量。当受试物的 LD50 大于 5g/kg 体重时，可取 5g/kg 体重为最高剂量。另外设阴性（溶剂）对照组和阳性物对照组。阳性对照组用环磷酰胺（40mg/kg 体重）或丝裂霉素 C（1.5mg/kg 体重—2mg/kg 体重），腹腔注射。每组至少有 5 只存活动物。

根据受试物的理化性质（水溶性和/或脂溶性）确定受试物所用的溶剂，通常用水、植物油或食用淀粉等。

8 Dose grouping

There are at least three dose groups for the tested substance. Doses of 1/2, 1/5, 1/10 or 1/20LD50 were taken respectively. When the LD50 of the test substance is greater than 5g/kg body weight, 5g/kg body weight is preferable as the highest dose. In addition, negative (solvent) control group and positive control group were set up. Positive control group was injected intraperitoneally with cyclophosphamide (40mg/kg body weight) or mitomycin C(1.5mg/kg body weight-2 mg/kg body weight). There are at least 5 living animals in each group.

According to the physical and chemical properties (water-soluble and / or fat soluble) of the test substance, the solvent used for the test substance is usually water, vegetable oil or edible starch.

9 染毒途径和方式

染毒途径视实验目的而定，建议采用经口灌胃方式。每天 1 次染毒（如剂量过大时，一天内染毒数次也是可以的，但每次应间隔数小时），连续 5d。于第 1 次染毒后的第 12d—14d 将受试动物处死。处死动物前 6h，腹腔注射 0.04%秋水仙素溶液，剂量为 4mg/kg 体重。

9 Ways and means of poisoning

The route of poisoning depends on the purpose of the experiment, and oral gavage is recommended. Once a day (if the dose is too large, several times a day is OK, but the interval should be several hours), for 5 days. The animals are killed from the 12th to the 14th day after the first exposure. Six hours before the animals are killed, 0.04% colchicine solution is injected intraperitoneally at a dose of 4 mg / kg body weight.

10 试验方法

10.1 取材

取出两侧睾丸，去净脂肪，于生理盐水中洗去毛和血污，放入盛有适量 1% 柠檬酸三钠或 0.075mol/L 氯化钾溶液的小平皿中。

10.2 制片

10.2.1 低渗：以眼科镊撕开被膜，轻轻地分离曲细精管，加入 1%柠檬酸三钠溶液 10mL，用滴管吹打曲细精管，室温下静止 20min。

10.2.2 固定：仔细吸尽低渗液，加固定液（甲醇：冰乙酸＝3∶1）10 mL 固定。第一次不超过 15 min，倒掉固定液后，再加入新的固定液固定 20min 以上 。如在冰箱（0℃-4℃）过夜固定更好。

10.2.3 离心：吸尽固定液，加 60% 冰乙酸 1—2 mL，待大部分曲细精管软化完后，立即加入倍量的固定液，打匀、移入离心管，以 1000 r/min 离心 10 min 。

10.2.4 滴片：弃去大部分上清液，留下约 0.5—1.0 mL ，充分打匀制成细胞混悬液，将细胞混悬液均匀地滴于冰水玻片上。每个样本制得 2—3 张。空气干燥或微热烘干。

10.2.5 染色：用 1∶10 Giemsa 液（PH 6.8）染色 10min（根据室温染色时间不同），用蒸馏水冲洗、晾干。

10 test method

10.1 Draw materials

Take out the testicles on both sides, remove the fat, ish the hair and blood stains in normal saline, and put them into a small dish containing a proper amount of 1% trisodium citrate or 0.075mol/l potassium chloride solution.

10.2 Production

10.2.1 Hypotonic: tear the tunica with ophthalmic forceps, gently separate the seminiferous tubules, add 10ml of 1% trisodium citrate solution, blow the seminiferous tubules with dropper, and keep still at room temperature for 20min.

10.2.2 Fixation: carefully suck up the hypotonic solution, and add 10 ml of fixed solution (methanol: glacial acetic acid = 3:1) for fixation. For the first time, it shall not be more than 15 min. after pouring out the fixing liquid, add new fixing liquid for more than 20 min. Overnight fixation in refrigerator (0 ℃ - 4 ℃) would be better.

10.2.3 Centrifugation: suck up the fixed solution, add 1-2 ml of 60% glacial acetic acid, after most of the fine convoluted tubes are softened, immediately add multiple amounts of fixed solution, mix and transfer into the centrifuge tube, and centrifugate at 1000 R / min for 10 min.

10.2.4 Drop: discard most of the supernatant, leave about 0.5-1.0ml, mix well to make cell suspension, and drop the cell suspension evenly on the ice water slide. Make 2-3 pieces of each sample. Air drying or slight heat drying.

10.2.5 Dyeing: dye with 1:10 Giemsa solution (pH 6.8) for 10min (according to different dyeing time at room temperature), wash with distilled water and dry in the air.

10.4 阅片

10.4.1 阅片要求

在低倍镜下按顺序寻找背景清晰、分散良好、染色体收缩适中的中期分裂相，然后在油镜下进行分析。由于低渗等机械作用的破坏，会导致处于中期的染色体发生丢失，所以，观察的中期相染色体数目应是 n 对双价体，每只动物至少分析 100 个中期分裂相的初级精母细胞。计数的细胞应含染色体数为 1n＋1 的中期相细胞。有对于畸变细胞还应记录显微镜视野的坐标位置及畸变类型。

10.4.2 染色体分析

除了可见到裂隙、短片、微小体外，还要分析互相易位、X-Y 和常染色体的单价体

10.4 Observation

10.4.1 Observation requirements

At low magnification, the metaphase with clear background, good dispersion and moderate chromosome contraction is found in sequence, and then analyzed under oil microscope. Due to the destruction of low permeability and other mechanical effects, the metaphase chromosomes will be lost. Therefore, the number of metaphase chromosomes observed should be n-pair bivalents. Each animal should analyze at least 100 primary spermatocytes of metaphase. The cells counted should contain the metaphase cells with chromosome number of 1n + 1.For aberrant cells, we should also record the coordinate position and aberrance type of microscope field.

10.4.2 Chromosome analysis

In addition to cracks, short, and tiny bodies, the univalent of translocation, X-Y, and autosomes should be analyzed

11 数据处理和结果判断

所得各组染色体畸变率用 χ2 检验，或其他适当的显著性检验方法进行统计学处理。当各剂量组与阴性(溶剂)对照组相比，畸变细胞率有显著性意义的增加，并有剂量-反应关系时；或仅一个剂量组有显著性意义的增加，经重复试验证实后，可判为试验结果阳性。

11 Data processing and result judgment

The chromosome aberration rate of each group is statistically analyzed by χ 2 test or other appropriate significance test. When there is a significant increase in the rate of aberrant cells and a dose-response relationship between each dose group and the negative (solvent) control group, or only one dose group has a significant increase, it can be judged as a positive test result after the retest verification.

12 结果解释

阳性结果证明受试物具有引起该种动物睾丸生殖细胞染色体畸变的能力。

阴性结果表明在本试验条件下受试物不引起该种动物睾丸生殖细胞染色体畸变。

12 Interpretation of results

The positive results showed that the test substance had the ability to cause chromosomal aberrations in testicular germ cells of this kind of animal.

The negative results showed that the test substance did not cause chromosomal aberrations in the testicular germ cells of this kind of animal.

14 亚慢性经口毒性试验

Subchronic Oral Toxicity Test

1 范围

本规范规定了啮齿类动物亚慢性经口毒性试验的基本原则、要求和方法。本规范适用于检测化妆品原料的亚慢性经口毒性。

2 试验目的

在估计和评价化妆品原料的毒性时，获得受试物急性毒性资料后，还需进行亚慢性经口毒性试验。通过该试验不仅可获得一定时期内反复接触受试物后引起的健康效应、受试物作用靶器官和受试物体内蓄积能力资料，并可估计接触的无有害作用水平，后者可用于选择和确定慢性试验的接触水平和初步计算人群接触的安全性水平。

14 Subchronic oral toxicity test

1 Range

This specification specifies the basic principles, requirements and methods of subchronic oral toxicity test for rodents. This specification is applicable to the detection of subchronic oral toxicity of cosmetic raw materials.

2 Test purpose

When estimating and evaluating the toxicity of cosmetic raw materials, subchronic oral toxicity test should be carried out after obtaining the acute toxicity data of the test substance. Through this experiment, we can not only obtain the data of health effect, target organ and accumulation capacity of the test object caused by repeated contact with the test object in a certain period of time, but also estimate the non harmful level of contact. The latter can be used to select and determine the contact water level of the chronic experiment and preliminarily calculate the safety level of human contact.

3 定义

3.1 亚慢性经口毒性 subchronic oral toxicity

是指在实验动物部分生存期内，每日反复经口接触受试物后所引起的不良反应。

3.2 未观察到有害作用的剂量水平 no observed adverse effect level(NOAEL）

在规定的试验条件下，用现有的技术手段或检测指标未观察到任何与受试物有关的毒性作用的最大剂量。

3.3 观察到有害作用的最低剂量水平 Lowest observed adverse effect level(LOAEL)

在规定的试验条件下，受试物引起实验动物组织形态、功能、生长发育等有害效应的最低剂量。

3.4 靶器官 Target organ

实验动物出现由受试物引起的明显毒性作用的器官。

3 Definition

3.1 Subchronic oral toxicity

It refers to the adverse reactions caused by repeated oral contact with the test substance every day during the partial survival period of the experimental animal.

3.2 No observed adverse effect level (NOAEL)

Under the specified test conditions, no maximum dose of toxic effect related to the test substance is observed by using the existing technical means or detection indicators.

3.3 Lowest observed adverse effect level (LOAEL)

Under the specified test conditions, the lowest dose of test substance causing harmful effects such as tissue morphology, function, growth and development of experimental animals.

3.4 Target organ

The organs of experimental animals with obvious toxic effects caused by the test substance.

4 试验的基本原则

以不同剂量受试物每日经口给予各组实验动物，连续染毒 90d，每组采用一个染毒剂量。染毒期间每日观察动物的毒性反应。在染毒期间死亡的动物要进行尸检。染毒结束后所有存活的动物均要处死，并进行尸检以及适当的病理组织学检查。

4 Basic principles of test

The experimental animals in each group are given different doses of the test substance orally every day for 90 days, and each group is given one dose. The toxic reaction of animals is observed every day during the period of exposure. Necropsy is required for animals that die during exposure. At the end of the exposure, all the surviving animals are killed and necropsy and appropriate histopathological examination are carried out.

5 试验方法

5.1 实验动物和饲养环境

5.1.1 动物种系的选择

常规选择啮齿类动物，首选大鼠。一般选用 6 周—8 周龄的大鼠。动物体重的变动范围不应超出平均动物体重的 20%。若该试验为慢性试验的预备试验，则在两个试验中所用的动物种系应当相同。

5.1.2 动物的性别和数量

每一剂量组实验动物至少应有 20 只（雌雄各半），但是考虑到亚慢性试验的重要性，应适当增加每组雌雄动物数。若计划在试验过程中处死动物，则应增加计划处死的动物数。试验结束时的动物数需达到能够有效评价受试物毒性作用的数量。此外，可另设一追踪观察组，选用 20 只动物（雌雄各半），给予最高剂量受试物，染毒 90d，在全程染毒结束后继续观察一段时间（一般不少于 28d），以了解毒性作用的持续性、可逆性或迟发毒作用。

5.1.3 饲养环境

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Laboratory animals and feeding environment

5.1.1 Selection of animal species

Rodents are selected as the first choice. Generally, rats aged 6-8 weeks are selected. The variation range of animal weight should not exceed 20% of the average animal weight. If the test is a preliminary test for a chronic test, the animal strains used in both tests should be the same.

5.1.2 Sex and number of animals

Each dose group should have at least 20 experimental animals (half male and half female), but considering the importance of subchronic tests, the number of male and female animals in each group should be appropriately increased. If it is planned to kill animals during the experiment, the number of animals to be killed should be increased. At the end of the test, the number of animals should reach the number that can effectively evaluate the toxicity of the tested substance. In addition, another follow-up observation group can be set up. 20 animals (half male and half female) are selected and given the highest dose of the tested substance for 90 days, and the observation period (generally not less than 28 days) is continued after the whole course of exposure to understand the persistence, reversibility or delayed toxicity of the toxic effect.

5.1.3 Feeding environment

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, drinking water is not limited.

5.2 剂量分组

试验时至少要设三个染毒组和一个对照组。除不接触受试物外，对照组的其他条件均与试验组相同。最高染毒剂量的设计应在引起中毒效应的前提下又不致造成动物过多死亡，否则将会影响结果的评价。低剂量组应不出现任何毒性作用。若掌握人群接触水平，则最低染毒剂量应高于人群的实际接触水平。中间剂量组应引起较轻的可观察到的毒性作用。若设多个中间剂量组，则各组的染毒剂量应引起不同程度毒性作用。在中、低剂量组和对照组中，动物死亡率应很低，以保证得到有意义的评价结论。

对那些毒性较低的物质来说，当通过饲料染毒时应特别注意确保大量的受试物混入不会对动物正常营养产生影响。对其他的染毒方式要加以特殊说明。若采用灌胃方式染毒，则每日染毒时点应相同，并定期（每周）按体重调整染毒剂量，维持单位体重染毒水平不变。

本项试验中，如果接触水平超过 1000mg/kg 时仍未产生可观测到的毒性效应，而且可以根据相关结构化合物预期受试物毒性时，可以考虑不必进行三个剂量水平的全面试验观察。

5.2 Dose grouping

At least three exposure groups and one control group should be set up in the experiment. The other conditions of the control group are the same as those of the test group except for the non-contact with the test substance. The design of the maximum dose should not lead to excessive death of animals on the premise of toxic effect, otherwise it will affect the evaluation of the results. There should be no toxic effect in the

low dose group. If the exposure level of the population is known, the lowest exposure dose should be higher than the actual exposure level of the population. The intermediate dose group should cause less observable toxicity. If there are more than one middle dose group, the dose of each group should cause different degrees of toxicity. In the middle and low dose group and the control group, the mortality rate of animals should be very low, so as to ensure a meaningful evaluation conclusion.

For those substances with low toxicity, special attention should be paid to ensure that a large number of test substances will not affect the normal nutrition of animals when they are exposed to feed. Other ways of poisoning should be specified. If the drug is administered by gavage, the time point of daily administration should be the same, and the dose should be adjusted regularly (weekly) according to the body weight to maintain the same level of unit body weight.

In this test, if the exposure level is more than 1000mg / kg, there is no observed toxic effect, and the toxicity of the test substance can be expected according to the related structural compounds, it can be considered that there is no need to conduct three dose levels of comprehensive test observation.

5.3 试验步骤

染毒开始前至少要有 5d 时间使实验动物适应实验室饲养环境。实验动物随机分组。受试物可通过混入饲料或饮水、直接喂饲以及灌胃进行染毒。动物每周 7d 染毒。试验期间所有动物染毒的方式应完全相同。若为染毒目的加入其他溶剂或添加剂，这些溶剂或添加剂不应影响受试物的吸收或引起毒性作用。

5.3 Test procedure

There should be at least 5 days before the start of poisoning to make the experimental animals adapt to the laboratory feeding environment. The experimental animals are randomly divided into groups. The test substance can be poisoned by mixing feed or drinking water, direct feeding and gavage. Animals are poisoned 7 days a week. All animals should be exposed in exactly the same way during the test. If other solvents or additives are added for the purpose of poisoning, they shall not affect the absorption of the test substance or cause toxicity.

5.4 临床观察

观察时间应至少为 90d。追踪观察组还要增加 28d，但不作任何处理，以了解毒性作用的可逆性、持续性及迟发毒作用。

观察期间对动物的任何毒性表现均应记录，记录内容包括发生时间、程度和持续时间。观察应至少包括如下内容：皮肤和被毛的改变、眼和粘膜变化、呼吸、循环、植物神经和中枢神经系统、肢体运动和行为活动等改变。应计算每周饲料消耗量（或当通过饮水染毒时的饮水消耗量），记录每周体重变化。

5.4 clinical observation

The observation time should be at least 90 days. In the follow-up observation group, 28 days are added, but no treatment is done to understand the reversibility, persistence and delayed toxicity of the toxicity.

Any toxicity to animals during the observation period shall be recorded, including the occurrence time, degree and duration. The observation should at least include the following contents: changes of skin and coat, changes of eyes and mucosa, changes of breath, circulation, autonomic and central nervous system, changes of limb movement and behavior. The weekly feed consumption (or water consumption when poisoned by drinking water) shall be calculated and the weekly weight change shall be recorded.

5.5 临床检查

5.5.1 眼科检查

在动物染毒前和染毒后，最好对所有实验动物，至少应对最高剂量组和对照组动物，使用眼科镜或其他有关设备进行眼科检查。若发现动物有眼科变化则应对所有动物进行检查。

5.5.2 血液检查

在染毒前、染毒中期、染毒结束及追踪观察结束时应测定血球容积、血红蛋白浓度、红细胞数、白细胞总数和分类，必要时测定凝血功能如凝血时间、凝血酶原时间、凝血激酶时间或血小板数等指标。

5.5.3 临床血液生化检查

在染毒前、染毒中期、染毒结束及追踪观察结束时进行，检查指标包括电解质平衡、碳水化合物代谢、肝、肾功能。可根据受试物作用形式选择其他特殊检查。推荐的指标包括：钙、磷、氯、钠、钾、禁食血糖（不同动物品系采用不同的禁食期）、血清谷丙转氨酶、血清谷草转氨酶、鸟氨酸脱羧酶、g 谷氨酰转肽酶、尿素氮、白蛋白、血液肌酐、总胆红素及总血清蛋白。必要时可进行脂肪、激素、酸碱平衡、正铁血红蛋白、胆碱酯酶活性的分析测定。此外，还可根据所观察到的毒性作用进行其他更大范围的临床生化检查，以便进行全面的毒性评价。

5.5 clinical examination

5.5.1 Ophthalmic examination

Before and after the animals are poisoned, it is better to use ophthalmoscope or other relevant equipment for ophthalmic examination on all experimental animals, at least the animals in the highest dose group and the control group.If eye changes are found in animals, all animals should be examined.

5.5.2 Blood test

The blood cell volume, hemoglobin concentration, the number of red blood cells, the total number of white blood cells and their classification should be measured before, during, after and at the end of follow-up observation. If necessary, the coagulation function such as coagulation time, prothrombin time, coagulation kinase time or platelet number should be measured.

5.5.3 Clinical blood biochemical examination

Before, during, at the end of exposure and at the end of follow-up observation, the examination indexes include electrolyte balance, carbohydrate metabolism, liver and kidney function. Other special examinations can be selected according to the acting form of the test object. The recommended indexes include: calcium, phosphorus, chlorine, sodium, potassium, fasting blood glucose (different fasting periods are adopted for different animal strains), serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, ornithine decarboxylase, G glutamyl transpeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein. When

necessary, fat, hormone, acid-base balance, ferrohemoglobin and cholinesterase activity can be analyzed and determined. In addition, other clinical biochemical tests in a wider range can be carried out according to the observed toxic effects so as to carry out a comprehensive toxicity evaluation.

5.5.4 尿液检查

一般不需要进行，只有当怀疑存在或观察到相关毒性作用时方需进行尿液检查。

5.6 病理检查

5.6.1 大体尸检

所有动物均应进行全面的大体尸检，内容包括动物的外观、所有孔道，胸腔、腹腔及其内容物。肝、肾、肾上腺、睾丸、附睾、子宫、卵巢、胸腺、脾、脑和心脏应在分离后尽快称重以防水分丢失。应将下列组织和器官保存在固定液中，以备日后进行病理组织学检查：所有大体解剖呈现异常的器官、脑（包括延髓/脑桥、小脑和大脑皮层、脑垂体）、甲状腺/甲状旁腺、胸腺、肺/气管、心脏、主动脉、唾液腺*、肝、脾、肾、肾上腺、胰、性腺、子宫、生殖附属器官*、皮肤*、食管、胃、十二指肠、空肠、回肠、盲肠、结肠、直肠、膀胱、前列腺、有代表性的淋巴结、雌性乳腺*、大腿肌肉*、周围神经、胸骨（包括骨髓）、眼*、股骨（包括关节面）*、脊髓（包括颈部、胸部、腰部）*和泪腺*。

* 只有当毒性作用提示或作为被研究的靶器官时才需要检查这些器官。

5.5.4 Urinalysis

Generally, it is not necessary to carry out a urine test only when the presence or observation of related toxic effects is suspected.

5.6 Pathological examination

5.6.1 General autopsy

All animals should undergo a comprehensive gross autopsy, including the animal's appearance, all channels, chest cavity, abdominal cavity and its contents. Liver, kidney, adrenal gland, testis, epididymis, uterus, ovary, thymus, spleen, brain and heart should be weighed as soon as possible after separation to prevent water loss. The following tissues and organs shall be preserved in the fixative. For future histopathological examination: all organs with gross anatomical abnormalities, brain (including medulla oblongata/pons, cerebellum and cerebral cortex, pituitary gland), thyroid gland/parathyroid gland, thymus gland, lung/trachea, heart, aorta, salivary gland *, liver, spleen, kidney, adrenal gland, pancreas, gonad, uterus, reproductive accessory organs *, skin *, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, prostate, Representative lymph nodes, female mammary glands *, thigh muscles *, peripheral nerves, sternum (including bone marrow), eyes *, femur (including articular surface) *, spinal cord (including neck, chest, waist) *, and lacrimal gland *.

* These organs need to be examined only when toxic effects are indicated or when they are target organs to be studied.

5.6.2 病理组织学检查

应对下述器官和组织进行检查：

(1) 所有最高剂量组和对照组动物的重要的和可能受到损伤的器官或组织，如高剂量组动物的器官或组织有病理组织学的病变，则应扩展至其他剂量组的相应的器官和组织。

(2) 各剂量组大体解剖见有异常的器官或组织。

(3) 其他剂量组动物的靶器官。

(4) 在追踪观察组，应对那些在染毒组呈现毒性作用的组织和器官进行检查。

5.6.2 Histopathological examination

The following organs and tissues should be examined:

(1) All the important and possibly damaged organs or tissues of the highest dose group and the control group animals, such as the organs or tissues of the high dose group animals with pathological changes, should be extended to the corresponding organs and tissues of other dose groups.

(2) The gross anatomy of each dose group showed abnormal organs or tissues.

(3) Target organs of other dose groups.

(4) In the follow-up group, the tissues and organs with toxic effects in the exposed group should be examined.

6 试验结果的评价

6.1 结果的处理

可通过表格形式总结试验结果，显示试验开始时各组动物数、出现损伤的动物数、损伤的类型和每种损伤的动物百分比。对所有数据应采用适当的统计学方法进行评价，统计学方法应在试验设计时确定。

6.2 结果评价

亚慢性经口毒性试验结果应结合前期试验结果，并考虑到毒性效应指标和尸检及病理组织学检查结果进行综合评价。毒性评价应包括受试物染毒剂量与是否出现毒性反应、毒性反应的发生率及其程度之间的关系。这些反应包括行为或临床异常、肉眼可见的损伤、靶器官、体重变化情况、死亡效应以及其他一般或特殊的毒性作用。在综合分析的基础上得出 90 天经口毒性的 LOAEL 和（或）NOAEL，为慢性毒性试验的剂量、观察指标的选择提供依据。

6 Evaluation of test results

6.1 Treatment of results

The test results can be summarized in tabular form, showing the number of animals in each group at the beginning of the test, the number of animals with injury, the type of injury and the percentage of animals with each injury. All data should be evaluated using appropriate statistical methods, which should be determined at the time of trial design.

6.2 Result evaluation

The results of subchronic oral toxicity test should be combined with the results of previous tests, and the toxicity index and the results of autopsy and histopathology should be considered for comprehensive evaluation. The toxicity evaluation should include the relationship between the dose of the test substance and whether there is toxic reaction, the incidence and degree of toxic reaction. These reactions include behavioral or clinical abnormalities, visible damage to the naked eye, target organs,

weight changes, death effects, and other general or special toxic effects. Based on the comprehensive analysis, the LOAEL and / or NOAEL of 90 day oral toxicity are obtained, which provided the basis for the selection of dose and observation index of chronic toxicity test.

7 试验结果的解释

亚慢性经口毒性试验能够提供受试物在经口反复接触时的毒性作用资料。其试验结果可在很有限的程度上外推到人，但它可为确定人群的允许接触水平提供有用的信息。

7 Interpretation of test results

Subchronic oral toxicity test can provide the toxicity data of the tested substance in repeated oral exposure. The test results can be extrapolated to human to a very limited extent, but it can provide useful information for determining the allowable exposure level of the population.

15 亚慢性经皮毒性试验

Subchronic Dermal Toxicity Test

1 范围

本规范规定了啮齿类动物亚慢性经皮毒性试验的基本原则、要求和方法。本规范适用于检测化妆品原料的亚慢性经皮毒性。

2 试验目的

在估计和评价化妆品原料的毒性时，获得受试物急性经皮毒性资料后，还需进行亚慢性经皮毒性试验。通过该试验不仅可获得在一定时期内反复接触受试物后可能引起的健康影响资料，而且为评价受试物经皮渗透性、作用靶器官和慢性皮肤毒性试验剂量选择提供依据。

15 Subchronic Dermal Toxicity Test

1 Range

This specification specifies the basic principles, requirements and methods of subchronic percutaneous toxicity test for rodents. This specification is applicable to the detection of subchronic transdermal toxicity of cosmetic raw materials.

2 Test purpose

In the estimation and evaluation of the toxicity of cosmetic raw materials, the subchronic dermal toxicity test should be carried out after obtaining the acute dermal toxicity data of the test substance. Through this test, we can not only obtain the data of the possible health effects caused by repeated exposure to the test substance in a certain period of time, but also provide the basis for the evaluation of the percutaneous permeability of the test substance, the target organ and the dose selection of the chronic dermal toxicity test.

3 定义

3.1 亚慢性经皮毒性 subchronic dermal toxicity

是指在实验动物部分生存期内，每日反复经皮接触受试物后所引起的不良反应。

3.2 未观察到有害作用的剂量水平 no observed adverse effect level(NOAEL）

在规定的试验条件下，用现有的技术手段或检测指标未观察到任何与受试物有关的毒性作用的最大剂量。

3.3 观察到有害作用的最低剂量水平 lowest observed adverse effect level(LOAEL）

在规定的试验条件下，受试物引起实验动物组织形态、功能、生长发育等有害效应的最低剂量。

3.4 靶器官 target organ

实验动物出现由受试物引起的明显毒性作用的器官。

3 Definition

3.1 Subchronic dermal toxicity

It refers to the adverse reactions caused by repeated skin contact with the test substance every day during the partial survival period of the experimental animal.

3.2 No observed adverse effect level (NOAEL)

Under the specified test conditions, no maximum dose of toxic effect related to the test substance is observed by using the existing technical means or detection indicators.

3.3 Lowest observed adverse effect level (LOAEL)

Under the specified test conditions, the lowest dose of test substance causing harmful effects such as tissue morphology, function, growth and development of experimental animals.

3.4 Target organ

The organs of experimental animals with obvious toxic effects caused by the test substance.

4 试验的基本原则

以不同剂量受试物每日经皮给予各组实验动物，连续染毒 90d，每组采用一个染毒剂量。染毒期间每日观察动物的毒性反应。在染毒期间死亡的动物要进行尸检。染毒结束后对所有存活的动物均要处死，并进行尸检以及适当的病理组织学检查。

4 Basic principles of test

The experimental animals in each group are given different doses of the test substance by skin every day for 90 days, and each group is given one dose. The toxic reaction of animals is observed every day during the period of exposure. Necropsy is required for animals that

die during exposure. At the end of the exposure, all the surviving animals are killed, and necropsy and appropriate histopathological examination are carried out.

5 试验方法

5.1 受试物

若受试物为固体，应将其粉碎并用水（或适当的介质）充分湿润，以保证受试物与皮肤有良好的接触。若采用介质，应考虑该介质对受试物皮肤通透性的影响。液体受试物一般不用稀释。

5.2 实验动物和饲养环境

5.2.1 动物种系的选择

可采用成年大鼠、家兔或豚鼠进行试验，也可使用其他种属的动物。当亚慢性试验作为慢性试验的预备试验时，则在两项试验中所使用的动物种系应当相同。

5.2.2 动物的性别和数量

每一剂量组实验动物至少应有 20 只（雌雄各半），皮肤健康。若计划在试验过程中处死动物，则应增加计划处死的动物数。此外，可另设一追踪观察组，选用 20 只动物（雌雄各半），给予最高剂量受试物，染毒 90d，全程染毒结束后继续观察一段时间（一般不少于 28d），以了解毒性作用的持续性、可逆性或迟发毒作用。

5 test method

5.1 Test substance

If the test substance is solid, it shall be crushed and moistened with water (or appropriate medium) to ensure good contact between the test substance and the skin.If the medium is used, the influence of the medium on the skin permeability of the test object shall be considered. In general, the liquid test substance does not need to be diluted.

5.2 Laboratory animals and feeding environment

5.2.1 Selection of animal species

Adult rats, rabbits or guinea pigs can be used for the test, and other species of animals can also be used. When the subchronic test is used as the preliminary test of the chronic test, the animal species used in the two tests should be the same.

5.2.2 Sex and number of animals

There should be at least 20 animals (half male and half female) in each dose group with healthy skin. If animals are planned to be killed during the test, the number of animals planned to be killed shall be increased. In addition, a follow-up observation group can be set up to select 20 animals (half male and half female) and give them the highest dose of test substance for 90 days. After the whole course of exposure, continue to observe for a period of time (generally no less than 28 days) to understand the persistence, reversibility or delayed toxicity of toxicity.

5.2.3 饲养环境

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5.3 剂量分组

试验时至少要设三个染毒组和一个对照组。除不接触受试物外，对照组的其他条件均与试验组相同。最高染毒剂量的设计应在引起中毒效应的前提下又不致造成动物过多死亡，否则将会影响结果的评价。低剂量组应不出现任何毒性作用。若掌握人群接触水平，则最低染毒剂量应高于人群的实际接触水平。中间剂量组应引起较轻的可观察到的毒性作用。若设多个中间剂量组，则各组的染毒剂量应引起不同程度毒性作用。在中、低剂量组和对照组中，动物死亡率应很低，以保证得到有意义的评价结论。

若受试物引起严重的皮肤刺激效应，则应降低受试物的使用浓度，尽管这样可导致原来在高剂量下出现的其他毒性作用减弱或消失。若在试验早期动物的皮肤受到严重损伤，则有必要终止试验，并使用较低的浓度重新开始试验。

本项试验中，如果接触水平超过 1000mg/kg 时仍未产生可观测到的毒性效应，而且可以根据相关结构化合物预期受试物毒性时，可以考虑不必进行三个剂量水平的全面试验观察。

5.2.3 Feeding environment

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, drinking water is not limited.

5.3 Dose grouping

At least three exposure groups and one control group should be set up in the experiment. The other conditions of the control group are the same as those of the test group except for the non-contact with the test substance. The design of the maximum dose should not lead to excessive death of animals on the premise of toxic effect, otherwise it will affect the evaluation of the results. There should be no toxic effect in the low dose group. If the exposure level of the population is known, the lowest exposure dose should be higher than the actual exposure level of the population. The intermediate dose group should cause less observable toxicity. If there are more than one middle dose group, the dose of each group should cause different degrees of toxicity. In the middle and low dose group and the control group, the mortality rate of animals should be very low, so as to ensure a meaningful evaluation conclusion.

If the test substance causes severe skin irritation, the concentration of the test substance should be reduced, although this may lead to the weakening or disappearance of other toxic effects that originally occurred at high doses. If the skin of the animal is severely damaged in the early stage of the test, it is necessary to terminate the test and restart the test with a lower concentration.

In this test, if the exposure level is more than 1000mg / kg, there is no observed toxic effect, and the toxicity of the test substance can be expected according to the related structural compounds, it can be considered that there is no need to conduct three dose levels of comprehensive test observation.

5.4 试验步骤

动物在试验前至少要在实验室饲养环境中适应 5d 时间。染毒前 24h，将动物躯干背部染毒区的被毛剪掉或剃除。大约每周要对染毒部位去毛。在使用剪刀或剃刀进行去毛时应特别小心，以防损伤动物的皮肤从而引起皮肤通透性的改变。染毒部位的面积不应小于动物体表面积的 10%，应通过对动物体重的测定确定染毒部位的面积。若受试物毒性较大，则可相对减小染毒区域的面积，但受试物应尽可能薄而均匀地涂敷于整个染

毒区域。在染毒操作期间应使用玻璃纸和无刺激的胶带将受试物固定，以保证受试物与皮肤有良好的接触，并防止动物舔食。

在 90d 试验期间，实验动物每周 7d 每天染毒 6h。追踪观察组则要多进行 28d 观察，以了解毒性作用的持续性、可逆性及迟发毒作用。

5.4 Test procedure

Before the experiment, the animals should adapt to the laboratory environment for at least 5 days.24 hours before the exposure, the hairs in the exposed area on the back of the animal's trunk are cut off or shaved off. Hair should be removed from the infected area about every week. Special care should be taken when using scissors or razors for hair removal to prevent damage to the animal's skin that may cause changes in skin permeability. The area of the infected part shall not be less than 10% of the body surface area of the animal, and the area of the infected part shall be determined by the measurement of the weight of the animal. If the toxicity of the test substance is large, the area of the contaminated area can be relatively reduced, but the test substance should be applied to the whole contaminated area as thin and even as possible. During the poisoning operation, cellophane and non irritating tape shall be used to fix the test substance to ensure good contact between the test substance and the skin and prevent animals from licking.

During the 90-day test, the animals were exposed to the virus for 6 hours every day, 7 days a week. In the follow-up observation group, 28 days more observation was needed to understand the persistence, reversibility and delayed toxicity of toxic effects.

5.5 临床观察

试验中每天至少应进行一次仔细的临床检查。

观察期间对动物的任何毒性表现均应记录，记录内容包括发生时间、程度和持续时间。笼边观察应至少包括如下内容：皮肤和被毛的改变、眼和粘膜变化、呼吸、循环、植物神经和中枢神经系统、肢体运动和行为活动等改变。应计算每周饲料消耗量，记录每周体重变化。

5.6 临床检查

5.6.1 眼科检查

在动物染毒前和染毒后，最好对所有实验动物，至少应对最高剂量组和对照组动物，使用眼科镜或其他有关设备进行眼科检查。若发现眼科变化则应对所有动物进行检查。

5.6.2 血液检查

在染毒前、染毒中期、染毒结束及追踪观察结束时应测定包括血球容积、血红蛋白浓度、红细胞数、白细胞总数和分类、必要时测定凝血功能，如凝血时间、凝血酶原时间、凝血激酶时间或血小板数等指标。

5.5 clinical observation

Careful clinical examination should be carried out at least once a day in the trial.

Any toxicity to animals during the observation period shall be recorded, including the occurrence time, degree and duration. Cage side observation should at least include the following contents: changes of skin and coat, changes of eyes and mucosa,

changes of breath, circulation, autonomic and central nervous system, changes of limb movement and behavior. Weekly feed consumption should be calculated and weekly weight changes recorded.

5.6 clinical examination

5.6.1 Ophthalmic examination

Before and after the animals are poisoned, it is better to use ophthalmoscope or other relevant equipment for ophthalmic examination on all experimental animals, at least the animals in the highest dose group and the control group. If eye changes are found, all animals should be examined.

5.6.2 Blood test

Before, during, after and after exposure, blood cell volume, hemoglobin concentration, red blood cell number, total number and classification of white blood cells, and coagulation function, such as coagulation time, prothrombin time, thrombokinase time or platelet number, should be determined.

5.6.3 临床血液生化检查

染毒前、染毒中期、染毒结束及追踪观察结束时进行，检查指标包括电解质平衡、碳水化合物代谢、肝、肾功能。可根据受试物作用形式选择其他特殊检查。推荐的指标包括：钙、磷、氯、钠、钾、禁食血糖（不同动物品系采用不同的禁食期）、血清谷丙转氨酶、血清谷草转氨酶、鸟氨酸脱羧酶、g 谷氨酰转肽酶、尿素氮、白蛋白、血液肌酐、总胆红素及总血清蛋白。必要时可进行脂肪、激素、酸碱平衡、正铁血红蛋白、胆碱酯酶活性的分析测定。此外，还可根据所观察到的毒性作用进行其他更大范围的临床生化检查，以便进行全面的毒性评价。

5.6.4 尿液检查

一般不需要进行，只有当怀疑存在或观察到相关毒性作用时方需进行尿液检查。

5.6.3 Clinical blood biochemical examination

Before, during, after and at the end of follow-up observation, electrolyte balance, carbohydrate metabolism, liver and kidney functions are examined. Other special inspection can be selected according to the action form of test substance. The recommended indexes include: calcium, phosphorus, chlorine, sodium, potassium, fasting blood glucose (different fasting periods are adopted for different animal strains), serum GPT, serum GST, ornithine decarboxylase, g-glutamyltranspeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein. If necessary, fat, hormone, acid-base balance, methemoglobin and cholinesterase activity can be determined. In addition, a wider range of clinical biochemical tests can be carried out according to the observed toxic effects in order to carry out a comprehensive toxicity evaluation.

5.6.4 Urinalysis

Generally, it is not necessary to carry out a urine test only when the presence or observation of related toxic effects is suspected.

5.7 病理检查

5.7.1 大体尸检

所有动物均应进行全面的大体尸检，内容包括机体的外观、所有孔道，胸腔、腹腔及其内容物。肝、肾、肾上腺、睾丸、附睾、子宫、卵巢、胸腺、脾、脑和心脏应

在分离后尽快称重以防水分丢失。应将下列组织和器官保存在固定液中，以便日后进行病理组织学检查：所有大体解剖呈现异常的器官、脑（包括延髓/脑桥、小脑和大脑皮层、脑垂体）、甲状腺/甲状旁腺、胸腺、肺/气管、心脏、主动脉、唾液腺*、肝、脾、肾、肾上腺、胰、性腺、子宫、生殖附属器官*、皮肤、食管、胃、十二指肠、空肠、回肠、盲肠、结肠、直肠、膀胱、前列腺、有代表性的淋巴结、雌性乳腺*、大腿肌肉*、周围神经、胸骨（包括骨髓）、眼*、股骨（包括关节面）*、脊髓（包括颈部、胸部、腰部）*和泪腺*。

* 只有当毒性作用提示或作为被研究的靶器官时才需要检查这些器官。

5.7 Pathological examination

5.7.1 autopsy

All animals should undergo a comprehensive gross autopsy, including the animal's appearance, all channels, chest cavity, abdominal cavity and its contents. Liver, kidney, adrenal gland, testis, epididymis, uterus, ovary, thymus, spleen, brain and heart should be weighed as soon as possible after separation to prevent water loss. The following tissues and organs shall be preserved in the fixative. For future histopathological examination: all organs with gross anatomical abnormalities, brain (including medulla oblongata/pons, cerebellum and cerebral cortex, pituitary gland), thyroid gland/parathyroid gland, thymus gland, lung/trachea, heart, aorta, salivary gland *, liver, spleen, kidney, adrenal gland, pancreas, gonad, uterus, reproductive accessory organs *, skin *, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, prostate, Representative lymph nodes, female mammary glands *, thigh muscles *, peripheral nerves, sternum (including bone marrow), eyes *, femur (including articular surface) *, spinal cord (including neck, chest, waist) *, and lacrimal gland *.

5.7.2 病理组织学检查

应对下述器官和组织进行病理组织学检查：

(1) 所有最高剂量组和对照组动物的重要的和可能受到损伤的器官或组织，如高剂量组动物的器官或组织有病理组织学病变则应扩展至其他剂量组的相应的器官和组织。

(2) 各剂量组大体解剖见有异常的器官或组织。

(3) 其他剂量组动物的靶器官。

(4) 对追踪观察组，应对那些在染毒组呈现毒性作用的组织和器官进行检查。

5.7.2 Histopathological examination

The following organs and tissues shall be examined for histopathology:

(1) All the important and possibly damaged organs or tissues of the highest dose group and the control group animals, such as the organs or tissues of the high dose group animals with pathological changes, should be extended to the corresponding organs and tissues of other dose groups.

(2) The gross anatomy of each dose group showed abnormal organs or tissues.

(3) Target organs of other dose groups.

(4) For the follow-up observation group, the tissues and organs with toxic effects in the exposed group should be examined.

6 试验结果的评价

6.1 结果的处理

可通过表格形式总结试验结果，显示试验开始时各组动物数、出现损伤的动物数、损伤的类型和每种损伤的动物百分比。对所有数据应采用适当的统计学方法进行评价，统计学方法应在试验设计时确定。

6.2 试验结果的评价

亚慢性经皮毒性试验结果应结合前期试验结果，并考虑到毒性效应指标和尸检及病理组织学检查结果进行综合评价。毒性评价应包括受试物染毒剂量与是否出现毒性反应、毒性反应的发生率及其程度之间的关系。这些反应包括行为或临床异常、肉眼可见的损伤、靶器官、体重变化情况、死亡效应以及其他一般或特殊的毒性作用。在综合分析的基础上得出 90 天经皮毒性的 LOAEL 和（或）NOAEL，为慢性毒性试验的剂量、观察指标的选择提供依据。

6 Evaluation of test results

6.1 Treatment of results

The test results can be summarized in tabular form, showing the number of animals in each group at the beginning of the test, the number of animals with injury, the type of injury and the percentage of animals with each injury. All data should be evaluated using appropriate statistical methods, which should be determined at the time of trial design.

6.2 Evaluation of test results

The results of subchronic percutaneous toxicity test should be combined with the results of previous tests, and the toxicity index, autopsy and histopathological examination results should be considered for comprehensive evaluation. The toxicity evaluation should include the relationship between the dose of the test substance and whether there is toxic reaction, the incidence and degree of toxic reaction. These reactions include behavioral or clinical abnormalities, visible damage to the naked eye, target organs, weight changes, death effects, and other general or special toxic effects. Based on the comprehensive analysis, the LOAEL and / or NOAEL of 90 days' percutaneous toxicity are obtained, which provided the basis for the selection of dose and observation index of chronic toxicity test.

7 试验结果的解释

亚慢性经皮毒性试验能够提供受试物在经皮反复接触时的毒性作用资料。其试验结果可在很有限的程度上外推到人，但它可为确定人群的允许接触水平提供有用的信息。

7 Interpretation of test results

Subchronic percutaneous toxicity test can provide the toxicity data of the tested substance in repeated percutaneous contact. The test results can be extrapolated to human to a very limited extent, but it can provide useful information for determining the allowable exposure level of the population.

16 致畸试验

Teratogenicity Test

1 范围

本规范规定了动物致畸试验的基本原则，要求和方法。本规范用于检测化妆品原料的致畸性。

2 试验目的

检测妊娠动物接触化妆品原料后引起胎鼠畸形的可能性。

16 Teratogenicity test

1 Range

This specification specifies the basic principles, requirements and methods of animal teratogenesis test. This specification is used to detect the teratogenicity of cosmetic raw materials.

2 Test purpose

To detect the possibility of fetal rat deformity caused by the contact of pregnant animals with cosmetic materials.

3 定义

致畸性 Teratogenicity
在胚胎发育期引起胎仔永久性结构和功能异常的化学物质特性。

4 试验基本原则

在胚胎发育的器官形成期给妊娠动物染毒，在胎鼠出生前将妊娠动物处死，取出胎鼠检查其骨骼和内脏畸形。

3 Definition

Teratogenicity

Chemical properties that cause permanent structural and functional abnormalities of the fetus during embryonic development.

4 Basic principles of test

Pregnant animals are poisoned in the organogenesis stage of embryo development, and are killed before birth, and their skeleton and visceral deformities are examined.

5 试验方法

5.1 试剂

5.1.1 甲醛、冰乙酸、2，4，6-三硝基酚、氢氧化钾、甘油、水合氯醛、茜素红。

5.1.2 茜素红贮备液：茜素红饱和液，50% 乙酸饱和液 5.0 mL，甘油 10.0 mL，1% 水合氯醛 60.0 mL 混合，放入棕色瓶中。

5.1.3 茜素红应用液：取贮备液 3—5 mL，用 1—2g/100 mL 氢氧化钾液稀释至 1000 mL，存于棕色瓶中。

5.1.4 茜素红溶液：茜素红 0.1g，氢氧化钾 10g，蒸馏水 1000mL。

5.1.5 透明液 A：甘油 200mL，氢氧化钾 10g 蒸馏水 790mL.

5.1.6 透明液 B：甘油与蒸馏水等量混合。

5.1.7 固定液（Bouins 液）：2,4,6-三硝基酚（苦味酸饱和液）75 份、甲醛 20 份、冰乙酸 5 份

5 test method

5.1 reagent

5.1.1 Formaldehyde, glacial acetic acid, 2,4,6-trinitrophenol, potassium hydroxide, glycerin, chloral hydrate, alizarin red.

5.1.2 Alizarin red stock solution: alizarin red saturated solution, 50% acetic acid saturated solution 5.0ml, glycerin 10.0ml, 1% chloral hydrate 60.0ml mixed, put into a brown bottle.

5.1.3 Alizarin red application solution: take 3-5ml of stock solution, dilute to 1000ml with 1-2g / 100ml potassium hydroxide solution, and store in brown bottle.

5.1.4 Alizarin red solution: 0.1g alizarin red, 10g potassium hydroxide, 1000ml distilled water.

5.1.5 Transparent solution A: Glycerin 200ml, potassium hydroxide 10g distilled water 790ml

5.1.6 Transparent solution B: mix glycerin and distilled water in equal amount.

5.1.7 Fixed solution (bouins solution): 75 parts of 2,4,6-trinitrophenol (picric acid saturated solution), 20 parts of formaldehyde, 5 parts of glacial acetic acid

5.2 实验动物和饲养环境

动物选择：首选为健康的性成熟大鼠。

实验动物及实验动物房应符合国家相应规定。

5.3 剂量和分组

至少设三个剂量组，最高剂量应能引起母鼠某些毒性反应，但不应引起 10%以上动物的死亡。最低剂量不会出现可观察到的毒性反应。另设阴性对照组。每组至少 12 只孕鼠。当初次进行致畸试验或使用新的动物种属和品系时，必须同时设阳性对照组，阳性对照物可选用敌枯双、维生素 A 等。

5.2 Laboratory animals and feeding environment

Animal selection: the first choice is healthy sexually mature rats.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations.

5.3 Dose and grouping

There should be at least three dose groups. The highest dose should be able to cause some toxic reactions in female mice, but it should not cause more than 10% of animal deaths. There will be no observed toxicity at the lowest dose. Another negative control group is set up. At least 12 pregnant rats in each group. When the first teratogenesis test is carried out or new animal species and strains are used, a positive control group must be set up at the same time. The positive control materials can be diclofenac, vitamin A, etc.

5.4 试验步骤

5.4.1 “孕鼠”的检出和给受试物时间

雌鼠和雄鼠按 1：1（或 2：1）同笼，每日晨观察阴栓（或阴道涂片），查出阴栓（或精子）的当天定为孕期零天。如果 5 d 内没查出“受精鼠”，应调换雌鼠。检出的“受精鼠”按随机分组。在孕期 6d—15d，每天经口给予受试物。孕鼠于孕期 0、6、10、15 和 20 d 称重，并根据体重调整给受试物量。

5.4.2 孕鼠处死和一般检查

大鼠于妊娠第 20 d 处死。剖腹检查卵巢内黄体数，取出子宫，称重；检查活胎、早期吸收和死胎数。

5.4.3 活胎鼠检查

逐一记录胎鼠体重、体长、尾长、检查胎鼠外观有无异常，如头部有无脑膨出、露脑、小头、小耳、小眼、无眼和睁眼、兔唇、下颌裂，躯干部有无腹壁裂、脐疝、脊柱弯曲，四肢有无小肢、短肢、并趾、多趾、无趾等畸形，尾部有无短尾、卷尾、无尾、肛门有无闭锁。

5.4 Test procedure

5.4.1 Detection of "pregnant mice" and time of test substance administration

Female rats and male rats are caged in the same cage according to 1:1 (or 2:1). Observe the Yin suppository (or vaginal smear) every morning, and the day of finding out the Yin suppository (or sperm) is determined as the zero day of pregnancy. If no "fertilized rat" is found within 5 days, the female rat should be replaced. The detected "fertilized mice" are randomly divided into groups. During the 6-15 days of pregnancy, the test substance is given orally every day. Pregnant rats are weighed at 0, 6, 10, 15 and 20 days of pregnancy, and the amount of test substance is adjusted according to the weight.

5.4.2 Execution and general examination of pregnant rats

The rats are killed on the 20th day of pregnancy. The number of corpus luteum in the ovary is examined by laparotomy, the uterus is taken out and weighed; the number of live fetus, early absorption and stillbirth are examined.

5.4.3 Live fetal rat examination

Record the body weight, body length and tail length of fetal rats one by one, and check whether the appearance of fetal rats is abnormal, such as whether the head has encephalocele, exposed brain, small head, small ear, small eye, no eye and open eye, cleft lip and mandible, whether the trunk has abdominal wall crack, umbilical hernia, spinal curvature, whether the limbs have small limbs, short limbs, combined toes, multi toes, no toes and other deformities, whether the tail has short tail, curly tail, tailless tail and anal atresia.

5.4.4 胎鼠骨标本的制作与检查

将每窝 1/2 的活胎鼠放入 95%（V/V）乙醇中固定 2 周—3 周，取出胎仔（或可去皮、去内脏及脂肪）流水冲洗数分钟后放入 1g—2g/100mL 的氢氧化钾溶液内（至少 5 倍于胎仔体积）8h—72h，透明后放入茜素红应用液中染色 6h—48h，并轻摇 1—2 次/d，至头骨染红为宜。再放入透明液 A 中 1d—2d，放入透明液 B 中 2d—3d，待骨骼染红而软组织基本褪色后，可将标本放在甘油中保存。也可将胎鼠剥皮、去内脏及脂肪后，放入茜素红溶液染色，当天摇动玻璃瓶 2—3 次，待骨骼染成红色时为止。将胎鼠放入透明液 A 中 1—2 天，换到透明液 B 中 2—3 天。待胎鼠骨骼已染红，而软组织的紫红色基本褪色后，可将标本放在甘油中保存。（剥皮法）将标本放入小平皿中，用透射光源，在体视显微镜下作整体观察，然后逐步检查骨骼。测量囟门大小，矢状缝的宽度，头顶间骨及后头骨缺损情况，然后检查胸骨的数目，缺失或融合（胸骨为 6 个，骨化不全时首先缺第 5 胸骨、次为缺第 2 胸骨）。肋骨通常 12—13 对，常见畸形有融合肋、分叉肋、波状肋、短肋、多肋、缺肋、肋骨中断。

脊柱发育和椎体数目（颈椎 7 个，胸椎 12—13 个，腰椎 5—6 个，底椎 4 个，尾椎 3—5 个），有无融合、纵裂等。最后检查四肢骨。

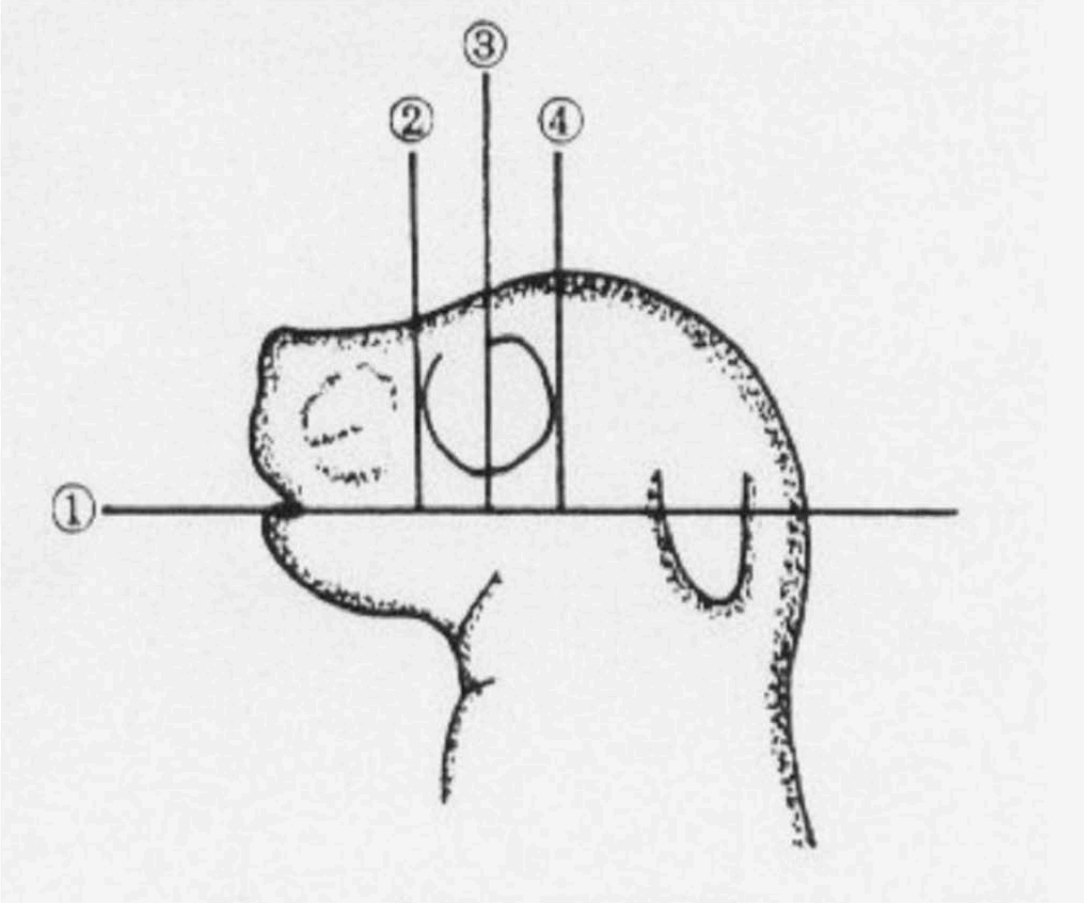
5.4.4 Preparation and examination of fetal rat bone specimen

Put 1 / 2 of each litter of live fetal rats into 95% (V / V) ethanol for 2-3 weeks, take out the fetus (or remove skin, viscera and fat) and wash it with running water for several minutes, then put it into 1G-2G / 100ml potassium hydroxide solution (at least 5 times the fetal volume) for 8h-72h, put it into alizarin red application solution for 6h-48h after being transparent, and gently shake it for 1-2 times / D until the skull is dyed red. Then put it into transparent solution a for 1d-2d, and put it into transparent solution B for 2D-3D. After the bone is dyed red and the soft tissue is basically faded, the specimen can be stored in glycerin. The fetal rats can also be peeled, viscera and fat removed, and then put into alizarin red solution for staining. Shake the glass bottle for 2-3 times on the same day until the bones are dyed red. The fetal rats are put into the transparent liquid A for 1 to 2 days, and changed into the transparent liquid B for 2 to 3 days. When the skeleton of fetal mouse has been dyed red and the purplish red of soft tissue has basically faded, the specimen can be stored in glycerin.(peeling method) put the specimen into a small plate, use a transmission light source, observe the whole body under a stereomicroscope, and then gradually check the skeleton. Measure the size of the fontanelle, the width of the sagittal suture, the defect of the

intercranial bone and the posterior skull, and then check the number of sternum, the defect or fusion (the number of sternum is 6, when the ossification is incomplete, the fifth sternum and the second sternum are missing first).rib
There are usually 12-13 pairs of bone, and the common deformities are fusion rib, bifurcate rib, undulate rib, short rib, multi rib, lack rib and rib interruption.
Spine development and number of vertebrae (7 cervical vertebrae, 12-13 thoracic vertebrae, 5-6 lumbar vertebrae, 4 bottom vertebrae, 3-5 tail vertebrae), fusion, longitudinal fissure, etc. Finally, examine the bone of limbs.

5.4.5 胎鼠内脏检查

每窝的 1/2 胎鼠放入 Bouins 液中，固定两周后作内脏检查。先用自来水冲去固定液，将鼠仰放在石蜡板上，剪去四肢和尾，用刀片从头部到尾部逐段横切或纵切。按不同部位的断面观察器官的大小、形状和相对位置。正常切面见图。



(1) 经口从舌与两口角向枕部横切（切面 1），观察大脑、间脑、延髓、舌及颚裂。
(2) 在眼前面作垂直纵切（切面 2），可见鼻部。
(3) 从头部垂直通过眼球中央作纵切（切面 3）。
(4) 沿头部最大横位处穿过作横切（切面 4）。
以上切面的目的可观察舌裂、颚裂、眼球畸形、脑和脑室异常。
(5) 沿下颚水平通过颈部中部作横切，可观察气管、食管和延脑或脊髓。

以后自腹中线剪开胸、腹腔，依次检查心、肺、横膈膜、肝、胃、肠等脏器的大小、位置，查毕将其摘除，再检查肾脏、输尿管、膀胱、子宫或睾丸位置及发育情况。然后将肾脏切开，观察有无肾盂积水与扩大。

5.4.5 Visceral examination of fetal rats

Each litter of 1 / 2 fetal rats is put into bouins solution and fixed for two weeks before visceral examination. First, flush out the fixing liquid with tap water, place the rat on the paraffin plate, cut off the limbs and tail, and use the blade to cut from the head to the tail section by section or lengthwise. The size, shape and relative position of organs are observed according to different sections. See figure for normal section.

(1) The brain, diencephalon, medulla oblongata, tongue and cleft jaw are observed.

(2) The nose can be seen by making a vertical longitudinal section (Section 2) in front of the eyes.

(3) The head is cut vertically through the center of the eyeball (Section 3).

(4) Cross cut (Section 4) along the maximum transverse position of the head.

The purpose of the above sections is to observe the cleft tongue, cleft jaw, malformation of eyeball, abnormality of brain and ventricle.

(5) The trachea, esophagus and medulla oblongata or spinal cord can be observed by transverse cutting along the middle part of the neck.

After that, cut the chest and abdomen from the midline of abdomen, check the size and position of heart, lung, diaphragm, liver, stomach, intestine and other organs in turn, remove them after checking, and then check the position and development of kidney, ureter, bladder, uterus or testis. Then the kidney is cut open to observe whether there is hydronephrosis and enlargement.

5.5 统计方法及结果评定

各种率的检查用 X2 检验，孕鼠增重用方差分析或非参数统计，胎鼠身长、体重、窝平均活胎数用 T 检验。结果应能得出受试物是否有母体毒性和胚胎毒性、致畸性，最好能得出最小致畸剂量。

为比较不同有害物质的致畸强度，可计算致畸指数，以致畸指数 10 以下为不致畸，10—100 为致畸，100 以上为强致畸。为表示有害物对人体危害的大小，可计算致畸危害指数，如指数大于 300 说明受试物对人危害小，100—300 为中等，小于 100 为危害大。

5.5 Statistical method and result evaluation

X2 test is used for the examination of various rates, ANOVA or nonparametric statistics are used for the increase of pregnant rats, and t test is used for the length, weight and average number of live fetuses.Results it should be able to find out whether the test substance has maternal toxicity, embryotoxicity and teratogenicity, and it is best to get the minimum teratogenicity dose.

In order to compare the teratogenic intensity of different harmful substances, the teratogenic index can be calculated. The teratogenic index below 10 is non-teratogenic, 10-100 is teratogenic, and over 100 is strongly teratogenic. In order to indicate the harm of harmful substances to human body, teratogenic harm index can be calculated. If the

index is greater than 300, the harm of the tested substance to human body is small, 100-300 is medium, and less than 100 is great.

$$\text{致畸指数} = \frac{\text{雌鼠}LD_{50}}{\text{最小致畸剂量}}$$

$$\text{致畸危害指数} = \frac{\text{最大不致畸剂量}}{\text{最大可能摄入量}}$$

Teratogenicity index= **Female LD50/** Minimum teratogenic dose

Teratogenic hazard index = Maximum non teratogenic dose/ Maximum possible intake dose

17 慢性毒性/致癌性结合试验

Combined Chronic Toxicity / Carcinogenicity Test

1 范围

本规范规定了动物慢性毒性/致癌性结合试验的基本原则、要求和方法。本规范适用于化妆品原料的慢性毒性和致癌性的检测。

17 Combined Chronic toxicity / carcinogenicity combination test

1 Range

This specification specifies the basic principles, requirements and methods of animal chronic toxicity / carcinogenicity combined test. This specification is applicable to the detection of chronic toxicity and carcinogenicity of cosmetic raw materials.

2 定义

2.1 慢性毒性 chronic toxicity

动物在正常生命期的大部分时间内接触受试物所引起的不良反应。

2.2 未观察到有害作用的剂量水平 no observed adverse effect level(NOAEL)

在规定的试验条件下，用现有的技术手段或检测指标未观察到任何与受试物有关的毒性作用的最大剂量。

2.3 观察到有害作用的最低剂量水平 lowest observed adverse effect level(LOAEL)

在规定的试验条件下，受试物引起实验动物组织形态、功能、生长发育等有害效应的最低剂量。

2.4 靶器官 target organ

实验动物出现由受试物引起的明显毒性作用的器官。

2.5 化学致癌物 chemical carcinogen

能引起肿瘤，或使肿瘤发生率增加的化学物。

2 Definition

2.1 Chronic toxicity

Adverse reactions caused by exposure of animals to the test substance for most of their normal life.

2.2 No observed adverse effect level (NOAEL)

Under the specified test conditions, no maximum dose of toxic effect related to the test substance is observed by using the existing technical means or detection indicators.

2.3 Lowest observed adverse effect level (LOAEL)

Under the specified test conditions, the lowest dose of test substance causing harmful effects such as tissue morphology, function, growth and development of experimental animals.

2.4 Target organ

The organs of experimental animals with obvious toxic effects caused by the test substance.

2.5 Chemical carcinogen

A chemical that causes or increases the incidence of tumors.

3 原理

化学物质在体内的蓄积作用，是发生慢性中毒的基础。慢性毒性试验是使动物长期地以一定方式接触受试物引起的毒性反应的试验。

当某种化学物质经短期筛选试验证明具有潜在致癌性，或其化学结构与某种已知致癌剂十分相近时，而此化学物质有一定实际应用价值时，就需用致癌性试验进一步验证。动物致癌性试验为人体长期接触该物质是否引起肿瘤的可能性提供资料。

3 principle

The accumulation of chemicals in the body is the basis of chronic poisoning. Chronic toxicity test is a test to make animals contact with the test substance in a certain way for a long time.

When a chemical substance is proved to have potential carcinogenicity by short-term screening test, or its chemical structure is very similar to that of a known carcinogen, and the chemical substance has certain practical application value, it needs to be further verified by carcinogenicity test. The animal carcinogenicity test provides data for the possibility of human body's long-term exposure to the substance to cause tumor.

4 试验的基本原则

在实验动物的大部分生命期间将受试化学物质以一定方式染毒，观察动物的中毒表现，并进行生化指标、血液学指标、病理组织学等检查，以阐明此化学物质的慢性毒性。

将受试化学物质以一定方式处理动物，在该动物的大部分或整个生命期间及死后检查肿瘤出现的数量、类型、发生部位及发生时间，与对照动物相比以阐明此化学物质有无致癌性。

4 Basic principles of test

During most of the life of experimental animals, the tested chemicals are poisoned in a certain way to observe the poisoning performance of animals, and the biochemical indexes, hematological indexes, histopathology and other tests are carried out to clarify the chronic toxicity of the chemicals.

The number, type, location and time of tumor occurrence are examined during most or the whole life of the animal and after death. Compared with the control animal, the carcinogenicity of the chemical substance is clarified.

5 实验动物和饲养环境

5.1 动物种类和品系的选择

为选择合适的动物（种类和品系），应该进行有关的急性、亚急性和毒物动力学试验。在评价致癌性时常用小鼠和大鼠，而进行慢性毒性试验常用大鼠和狗。

对慢性毒性/致癌性结合试验，一般均采用大鼠，但这并不排斥使用其他种类。所选用的品系应是对该类受试物的致癌和毒性作用敏感的。

5.2 性别和实验开始时的年龄

两种性别都应该使用，最常使用刚断奶或已断奶的年幼动物来进行慢性毒性和致癌性的长期生物学试验。

在啮齿类动物断奶和适应环境之后要尽快开始试验，最好在 6 周龄之前。

5 Laboratory animals and feeding environment

5.1 Selection of animal species and strains

In order to select suitable animals (species and strains), the related acute, subacute and toxicokinetic tests should be carried out. Mice and rats are commonly used in the evaluation of carcinogenicity, while rats and dogs are commonly used in the chronic toxicity test.

For chronic toxicity/carcinogenicity combination tests, rats are generally used, but this does not exclude the use of other types. The selected strain should be sensitive to carcinogenic and toxic effects of the tested substance.

5.2 Gender and age at the beginning of the experiment

The two genders should be used, and young animals just weaned or weaned are most often used for long-term biological tests of chronic toxicity and carcinogenicity.

Start the experiment as soon as possible after the rodents are weaned and acclimated, preferably before the age of 6 weeks.

5.3 实验组的动物数

应保证试验结果的可靠性并能进行统计学处理，实验组和对照组动物，应采用随机分配的方法。

每组都应有足够的动物数用来进行详细的生物学和统计学分析。

每一个剂量组和相应的对照组至少应该有 50 只雄性和 50 只雌性的动物，不包括提前剖杀的动物数。如需观察肿瘤以外的病理变化可设附加剂量组，两种性别各 20 只动物，其相应的对照组两种性别各 10 只动物。

5.4 动物的管理、饲料和饮水

必须严格的控制环境条件和合理的动物管理措施。实验动物及实验动物房应符合国家相应规定。

5.3 Number of animals in the experimental group

The reliability of the test results should be guaranteed and statistical treatment can be carried out. The animals in the experimental group and the control group should be randomly assigned.

Each group should have enough animals for detailed biological and statistical analysis.

There should be at least 50 males and 50 females in each dose group and corresponding control group, excluding the number of animals killed in advance. If pathological changes other than tumors need to be observed, additional dose groups can be set up, with 20 animals of each sex and 10 animals of each sex in the corresponding control group.

5.4 Animal management, feed and drinking water must be strictly control environmental conditions and take reasonable animal management measures. The laboratory animal and laboratory animal room shall comply with the relevant national regulations.

6 剂量组和给受试物的频率

为了评价致癌性试验，至少要设三个剂量组的实验组及一个相应的对照组。高剂量组可以出现某些较轻的毒性反应，但不能明显缩短动物寿命。这些毒性反应可能表现在血清酶水平的改变，或体重增加受到轻度抑制（低于 10%）。

低剂量不能引起任何毒性反应，应不影响动物的正常生长、发育和寿命。一般不应低于高剂量的 10%。

中剂量应界于高剂量和低剂量之间，可根据化学物的毒代动力学性质来确定。

结合慢性毒性试验，应附加一个实验组和相应的对照组。最高剂量应能产生明显的毒性。一般每天给予受试物。如果所给的化学物质是混在饮水中或饲料中，应保证连续给予。给受试物的频率也可以按其毒代动力学变化进行调整。

应设相应的对照组，除不接触受试物外，其他条件应和实验组相同。

6 Dose group and frequency of test substance administration

In order to evaluate the carcinogenicity test, at least three dose groups and a corresponding control group should be set up. In the high dose group, there are some mild toxic reactions, but the life span of animals could not be shortened obviously. These toxic reactions may be manifested in changes in serum enzyme levels or mild inhibition of weight gain (less than 10%).

Low dose can not cause any toxic reaction, and it should not affect the normal growth, development and life span of animals. Generally, it should not be less than 10% of the high dose.

The middle dose should be between high dose and low dose, which can be determined according to the toxicokinetic properties of chemicals.

Combined with chronic toxicity test, an experimental group and corresponding control group should be added. The highest dose should produce obvious toxicity. Subjects are generally given daily. If the given chemicals are mixed in drinking water or feed, continuous administration shall be ensured. The frequency of administration of the test substance can also be adjusted according to its toxicity kinetics.

A corresponding control group shall be set up, and the other conditions shall be the same as that of the experimental group except that the subjects are not contacted.

7 给受试物的途径

经口，经皮，吸入是三种主要给受试物途径。选择何种途径要根据受试物的理化特性和对人有代表性的接触方式。

给受试物的频率按所选择的给予途径和方式可以有所不同，如有可能，应按照受试物的毒代动力学变化进行调整。

7.1 经口染毒

如果受试物是通过胃肠道吸收的则最好选用经口途径。按试验期限（9）中指明的试验期限，把受试物混入饲料中、溶于饮水中，或用管饲法连续给予动物。每周 7 天均给予受试物，中断染毒可使动物得到恢复或毒性缓解，从而影响结果及以后的评价。

7.2 经皮染毒

选择皮肤接触方式是用于模仿人接触有关物质的一个主要途径，并作为诱发皮肤病变的试验模型。有关诱导皮肤肿瘤的特殊试验在本方法中不作介绍。

7.3 吸入染毒

吸入方式不是化妆品主要接触途径，因此吸入染毒本方法不作介绍。

7 Approach to test substance

Oral, dermal and inhalation are the three main ways to give the test substance. Which way to choose should be based on the physical and chemical characteristics of the test object and the representative contact way to people.

The frequency of administration of the test substance can be different according to the chosen administration route and method, and if possible, it should be adjusted according to the toxicity dynamics of the test substance.

7.1 Oral poisoning

If the test substance is absorbed through gastrointestinal tract, oral route is the best choice. According to the test period specified in test period (9), the test substance shall be mixed into feed, dissolved in drinking water, or continuously given to animals by tube feeding method. The test substance is given 7 days a week. Discontinuation of exposure could restore or alleviate the toxicity of the animal, thus affecting the results and subsequent evaluation.

7.2 Transdermal poisoning

The choice of skin contact mode is a main way to imitate human contact with related substances, and it is used as the experimental model to induce skin lesions. Special tests for the induction of skin tumors are not described in this method.

7.3 Inhalation exposure

Inhalation is not the main contact way of cosmetics, so this method is not introduced.

8 试验期限

在附加组中 20 只实验动物/每性别和 10 只相应对照组动物/每性别至少应该维持到 12 个月。这些动物的剖杀，应是用于评价和受试物有关的，但并非老年性改变所导致的病理变化。致癌性试验的期限必须包括受试物正常生命期的大部分时间。确定试验期限的几条准则：

（1）一般情况下，试验结束时间对小鼠和仓鼠应在 18 个月，大鼠在 24 个月；然而对某些生命期较长的或自发肿瘤率低的动物品系，小鼠和仓鼠可在 24 个月，大鼠可在 30 个月。

（2）当最低剂量和对照组存活动物只有 25%时，也可以结束试验。对于有明显性别差异的试验，则其结束的时间对不同的性别应有所不同。在某种情况下因明显的毒性作用只造成高剂量组动物过早死亡，此时不应结束试验。

8 Test period

In the additional group, 20 experimental animals/each sex and 10 corresponding control animals/each sex should be maintained for at least 12 months. The necropsy of these animals should be used to evaluate the pathological changes related to the test object, but not caused by senile changes. The duration of carcinogenicity test must include most of the normal life of the subject. Several Criteria for Determining Test Duration:

(1) In general, the end time of the test should be 18 months for mice and hamsters and 24 months for rats; However, for some animal strains with long life span or low spontaneous tumor rate, mice and hamsters can be within 24 months and rats can be within 30 months.

(2) When only 25% of the animals in the lowest dose and control group survived, the experiment could also be ended. For experiments with significant gender differences, the end time should be different for different genders. In some cases, the high dose group animals died prematurely due to the obvious toxic effect. At this time, the test should not be ended.

9 临床观察和检查

9.1 观察

至少每天进行一次动物情况的检查。每天还应有数次有目的的观察，如剖检死亡动物或存入冰箱，将有病或垂死的动物分开或处死。及时发现所有的毒性作用的开始及其变化，并能减少因疾病、自溶或被同类所食造成的动物损失。

详细记录动物的症状包括神经系统和眼睛的改变，可疑肿瘤在内的所有毒性作用出现和变化的时间，以及死亡情况。

在试验的前 13 周内，每周称量体重一次，以后每 4 周称量一次。在试验的前 13 周内，每周检查一次动物的食物摄取情况，以后如动物健康状况或体重无异常改变，则每 3 个月检查一次。

9 Clinical observation and examination

9.1 observation

Check the condition of animals at least once a day. There should be several times of purposeful observation every day, such as dissecting dead animals or storing them in the refrigerator, separating or killing sick or dying animals. To detect the beginning and change of all toxic effects in time, and reduce the loss of animals caused by diseases, autolysis or being eaten by the same kind.

Detailed records of animal symptoms including changes in the nervous system and eyes, the time of occurrence and change of all toxic effects including suspicious tumors, and death are made.

During the first 13 weeks of the test, the body weight was weighed once a week and every 4 weeks thereafter. During the first 13 weeks of the experiment, the food intake of the animals was checked once a week, and thereafter every 3 months if there was no abnormal change in the animal's health or body weight.

9.2 血液学检查

血液学检查（血红蛋白含量，血球压积，红血球计数，白血球计数，血小板，或其他血凝试验）应在 3 个月，6 个月，以后每隔 6 个月及实验结束时进行，各组每个性别要检查 20 只大鼠。每次采集的血标本应来自相同的大鼠。最高剂量组和对照组大鼠应在同样的时间间隔内进行白血球分类计数，中等剂量组大鼠只是在必要时才做。

在试验期间，如果大体观察表明动物健康恶化，应对有关动物进行血球分类计数检查。高剂量和对照组动物要进行血球分类计数。如两组间有很大差异时，应对较低剂量组的动物进行血球分类计数。

9.2 Hematology examination

Hematological examination (hemoglobin content, hematocrit, red blood cell count, white blood cell count, platelet, or other hemagglutination test) should be carried out in 3 months, 6 months, and then every 6 months and at the end of the experiment. 20 rats of each sex in each group should be examined. Each blood sample collected should be from the same rat. The rats in the highest dose group and the control group should be counted in the same time interval, and the rats in the middle dose group should only do it when necessary.

During the test, if the general observation shows that the health of the animals has deteriorated, the relevant animals shall be checked for blood cell classification and counting. The blood cells of high dose and control group should be classified and counted. If there is a big difference between the two groups, the blood cells in the lower dose group should be classified and counted.

9.3 尿分析

收集各组每性别 10 只大鼠尿样进行分析，最好是在做血液检查的同时并取自同一大鼠。应测下列指标，可单个进行，也可每组相同性别的尿标本混在一起测定。

分析指标：外观；每个动物的尿量和比重；蛋白，糖，酮体，潜血（半定量）；沉淀物镜检（半定量）。

9.3 Urinalysis

The urine samples of 10 rats of each sex in each group are collected for analysis, preferably at the same time of blood examination and taken from the same rat. The following indicators should be measured, either individually or by mixing urine samples of the same sex in each group.

Analysis indicators: appearance; urine volume and specific gravity of each animal; protein, sugar, ketone body, occult blood (semi quantitative); precipitation objective microscopy (semi quantitative).

9.4 临床化学

每 6 个月及实验结束时，收集各组每性别的 10 只大鼠的血液标本进行临床化学检查，尽可能在各个时间间隔内采取相同的大鼠血标本。分离血浆，进行下列指标测定：

总蛋白浓度；白蛋白浓度；肝功能试验（如碱性磷酸酶，谷丙转氨酶，谷草转氨酶，γ 谷氨酰转肽酶，鸟氨酸脱羧酶）；糖代谢，如糖耐量；肾功能，如血尿素氮。

9.4 Clinical chemistry

Every 6 months and at the end of the experiment, blood samples of 10 rats of each sex in each group are collected for clinical chemical examination, and the same blood samples of rats are taken as far as possible in each time interval. Plasma is separated and the following indexes are determined:

Total protein concentration; albumin concentration; liver function test (such as alkaline phosphatase, glutamic pyruvic transaminase, glutamic oxaloacetylase, gamma glutamyltranspeptidase, ornithine decarboxylase); glucose metabolism, such as glucose tolerance; renal function, such as blood urea nitrogen.

9.5 病理检查

肉眼和病理检查常常是慢性/致癌性结合试验的基础。

9.5.1 肉眼剖检

所有的动物包括那些在实验过程中死亡或因处于垂死状态而被处死的，应进行肉眼检查。在所有动物被处死前，应收集血样品进行血球分类计数。保存所有肉眼可见的肿瘤或可疑为肿瘤的。所有的器官或组织都应保留以进行镜下检查。一般包括下列器官和组织：

脑* （髓/脑桥，小脑皮质，大脑皮质），垂体，甲状腺（包括甲状旁腺），胸腺，肺（包括气管），心脏，唾液腺，肝*，脾，肾*，肾上腺*，食管，胃，十二指

肠，空肠，回肠，盲肠，结肠，直肠，膀胱，淋巴结，胰腺，性腺*，生殖附属器官，乳腺，皮肤，肌肉，外周神经，脊髓（颈，胸，腰），胸骨或股骨（包括关节）和眼。肺和膀胱用固定剂填充能更好地保存组织。

9.5 Pathological examination

Macroscopic and pathological examination are often the basis of the chronic / carcinogenic combination test.

9.5.1 Visual sectioning

All animals, including those who died in the course of the experiment or are put to death due to being in a dying state, should be examined with naked eyes. Blood samples should be collected for blood cell count before all animals are killed. Preserve all visible or suspected tumors. All organs or tissues should be preserved for microscopic examination. It generally includes the following organs and tissues:

Brain * (medulla/pontine, cerebellar cortex, cerebral cortex), pituitary gland, thyroid gland (including parathyroid gland), thymus gland, lung (including trachea), heart, salivary gland, liver *, spleen, kidney *, adrenal gland *, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, lymph node, pancreas, gonad *, reproductive accessory organs, breast, skin, muscle, peripheral nerve, spinal cord (neck, chest, waist), sternum or femur (including joint) and eye. Filling lung and bladder with fixative can better preserve tissue.

9.5.2 组织病理检查

所有肉眼可见的肿瘤和其他病变都应进行病理检查。此外还要注意下列方面：

（1）对所有保存的器官和组织进行镜下检查，详细描述发现的所有病变。

①包括实验过程中死亡或处死的动物。

②所有最高剂量组和对照组动物。

（2）在较低剂量组，由受试物引起或可能由受试物引起异常的器官或组织也应进行检查。

*啮齿动物每组每性别 10 只，非啮齿动物全部标有*号的器官包括甲状腺及甲状旁腺都应称重。

9.5.2 Histopathological examination

All visible tumors and other lesions should be examined by pathology. In addition, pay attention to the following aspects:

(1) All preserved organs and tissues are examined under microscope, and all lesions found are described in detail.

① Including animals that died or are executed during the experiment.

② All animals in the highest dose group and the control group.

(2) In the lower dose group, organs or tissues caused by or possibly caused by the test substance should also be examined.

*There are 10 rodents of each sex in each group. All organs marked with * in non rodents, including thyroid gland and parathyroid gland, should be weighed.

10 数据处理和结果评价

可通过表格形式总结试验结果，显示试验各时段各组动物数、出现病变的动物数、病变类型等。对所有数据应采用适当、合理的统计学方法进行评价，统计学方法应在试验设计时确定。

慢性毒性与致癌合并试验应结合前期试验结果，并考虑到毒性效应指标和解剖及组织病理学检查结果进行综合评价。结果评价应包括受试物慢性毒性的表现、剂量-反应关系、靶器官、可逆性，得出慢性毒性相应的 NOAEL 和（或）LOAEL。

10.1 肿瘤发生率

肿瘤的发生率是整个实验终了时患瘤动物总数在有效动物总数中所占的百分率。有效动物总数指最早出现肿瘤时的存活动物总数。必要时根据试验中动物死亡率来调整计算致癌率，计算方法可参考有关文献。

10 Data processing and result evaluation

The results of the test can be summarized in the form of tables, showing the number of animals in each group, the number of animals with pathological changes and the types of pathological changes in each period of the test.All data should be evaluated with appropriate and reasonable statistical methods, which should be determined at the time of test design.

The combined test of chronic toxicity and carcinogenesis should be combined with the results of previous tests, and the toxicity index, anatomical and histopathological examination results should be taken into account for comprehensive evaluation. Results the evaluation should include the performance of chronic toxicity, dose-response relationship, target organ, reversibility, and the NOAEL and / or LOAEL corresponding to chronic toxicity.

10.1 Tumor incidence

The incidence of tumor is the percentage of the total number of animals with tumor in the total number of effective animals at the end of the whole experiment. The total number of effective animals refers to the total number of living animals at the time of the earliest occurrence of tumor. If necessary, the carcinogenic rate should be adjusted according to the animal mortality in the experiment. The calculation method can refer to the relevant literature.

$$\text{肿瘤发生率} = \frac{\text{试验结束时患瘤动物总数}}{\text{有效动物总数}} \times 100\%$$

Tumor incidence rate= The total number of animals suffering from tumor at the end of the experiment/ Total number of animals valid x 100%

10.2 致癌试验阳性的判断标准

采用世界卫生组织提出的四条判断诱癌试验阳性的标准：

（1）肿瘤只发生在染毒组动物中，对照组无该类型肿瘤；

（2）染毒组与对照组动物均发生肿瘤，但剂量组发生率明显增高；

（3）染毒组动物中多发性肿瘤明显，对照组中无多发性肿瘤或只少数动物有多发性肿瘤；

（4）染毒组与对照组动物肿瘤的发生率无显著性差异，但染毒组中肿瘤发现的时间较早。

上述四条中，试验组与对照组之间的数据经统计学处理后任何一条有显著性差异即可认为该受试物的致癌试验为阳性结果。染毒组和对照组肿瘤发生率差别不明显，但癌前病变差别显著时，不能轻易否定受试物的致癌性。

10.3 致癌试验阴性结果的确立

假如动物实验的规模为两种种属、两种性别，至少 3 个剂量水平，其中一个接近最大耐受剂量，每组动物数至少 50 只，实验组肿瘤发生率与对照组无差异，才算阴性结果。

10.2 Criteria for positive carcinogenesis test

Four criteria proposed by the World Health Organization are used to judge the positive results of cancer induction test：

(1) The tumor only occurred in the animals in the exposure group, but not in the control group;

(2) The incidence of tumor in the dose group is significantly higher than that in the control group;

(3) In the control group, there are no multiple tumors or only a few animals had multiple tumors;

(4) There is no significant difference in the incidence of tumor between the treated group and the control group, but the tumor is found earlier in the treated group.

Among the above four items, if there is significant difference between the data of the test group and the control group after statistical processing, it can be considered that the carcinogenic test of the test substance is a positive result. The difference of tumor incidence between the exposure group and the control group is not obvious, but when the difference of precancerous lesions is significant, the carcinogenicity of the test substance could not be easily denied.

10.3 Establishment of negative result of carcinogenic test

If the scale of animal experiment is two genera, two genders, at least three dose levels, one of which is close to the maximum tolerance

The number of animals in each group is at least 50, and the incidence of tumor in the experimental group is no different from that in the control group, so the negative result is calculated.